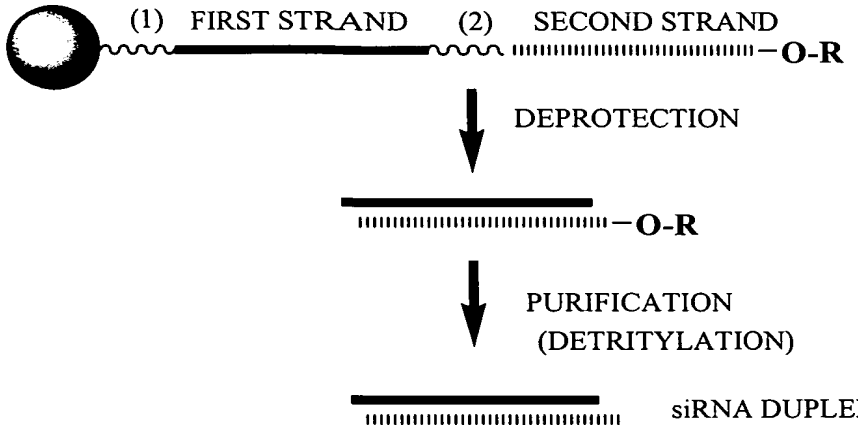
Figure 1

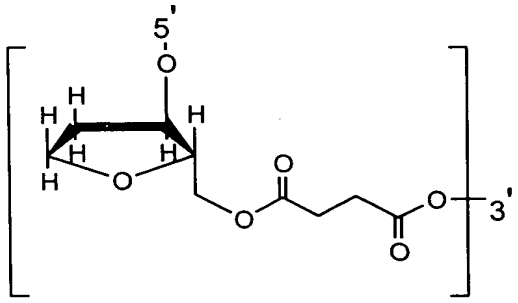


= SOLID SUPPORT

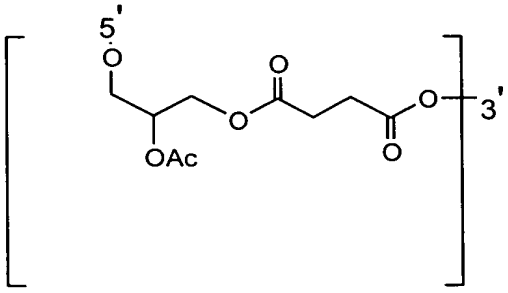
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

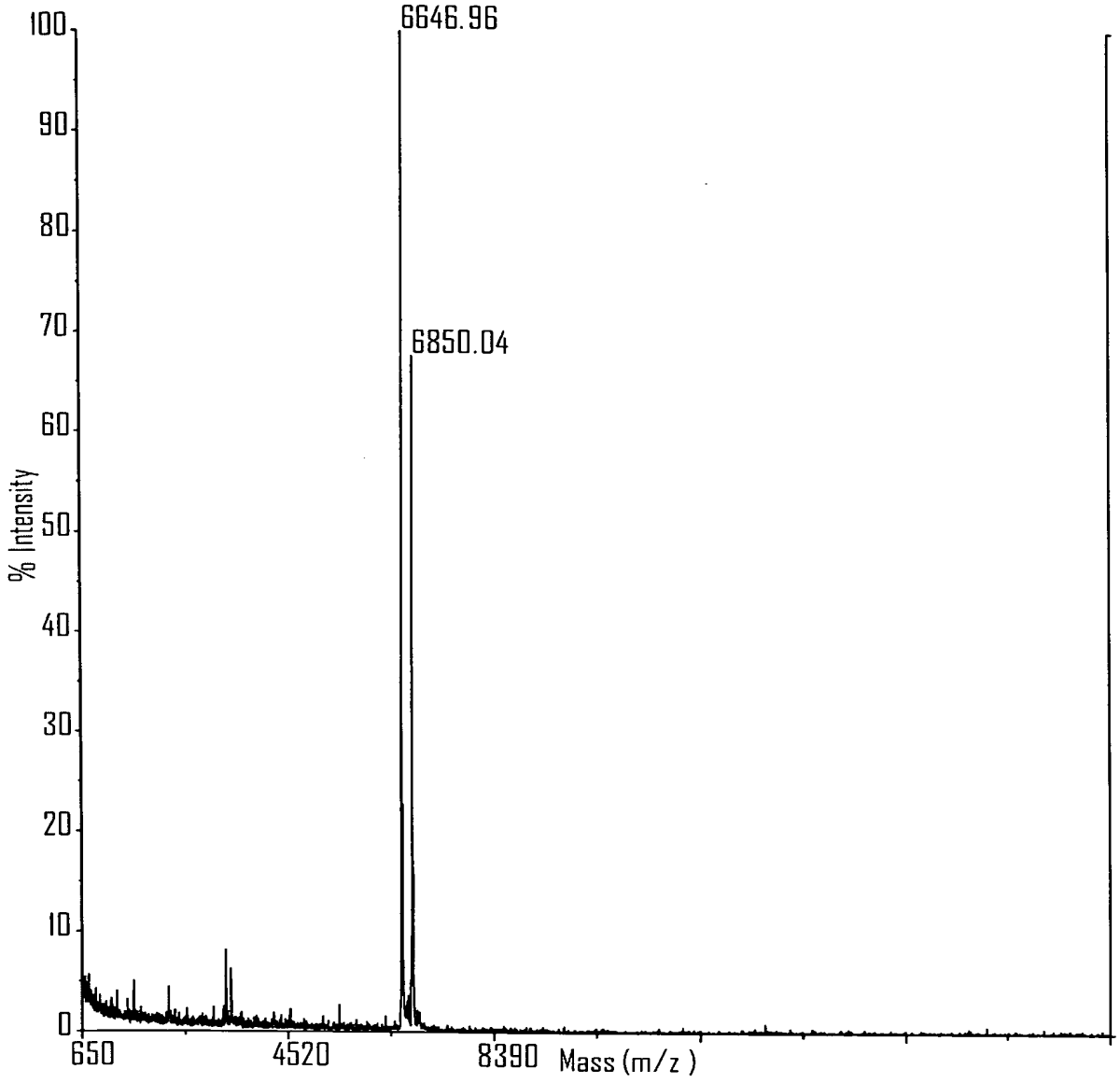

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

5'-CGUACGCGGAAUACUUCGATT (SEQ ID NO: 394) 3'-TTGCAUGCGCCUUAUGAAGCU (SEQ ID NO: 395)	T ½ = 15 seconds (control)
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-TXGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 397)	T ½ = 138 min
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-TDGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 398)	T ½ = 3.7 days
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396)	T ½ = 72 minutes
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396)	T ½ = 40 days
5'-B cAAccAcAAAAuAcAAcAATT B (SEQ ID NO: 396) 3'-tTGuuGGuGuuuuAuGuuGuu (SEQ ID NO: 401)	T ½ = 32 days

G, A, U, C = Guanosine, Adenosine, Uridine, Cytidine
T = Thymidine
Lower Case = 2'-deoxy-2'-fluoro
S = phosphorothioate
B = inverted deoxyabasic
G = terminal glycine
D = inverted Thymidine
X = 3'-deoxy Thymidine
t = L-thymidine
L = Glyceryl moiety

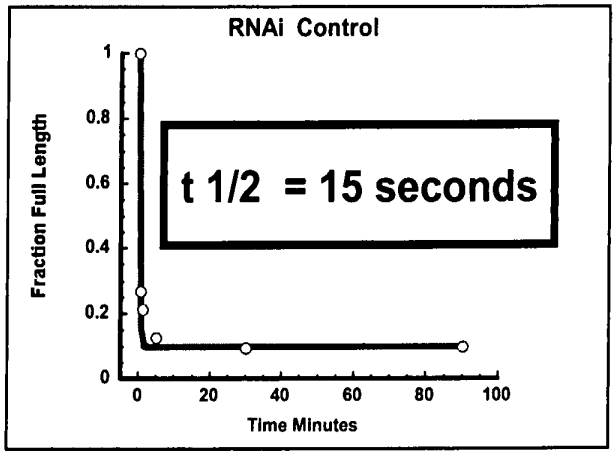


Figure 4

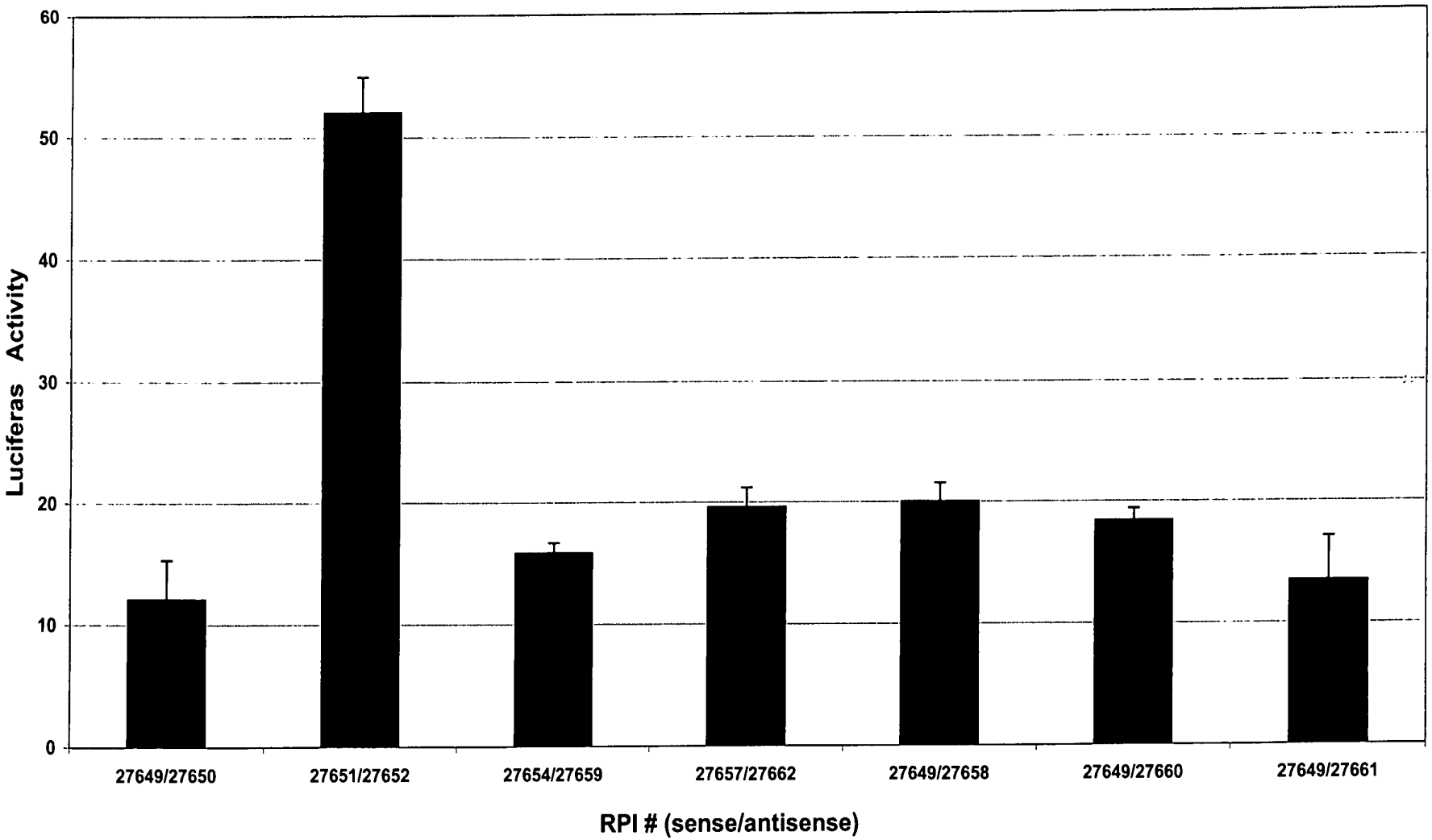

60
50
40
30
20
10
0
Luciferas Activity
27649/27650
27651/27652
27654/27659
27657/27662
27649/27658
27649/27660
27649/27661
RPI # (sense/antisense)

Figure 5

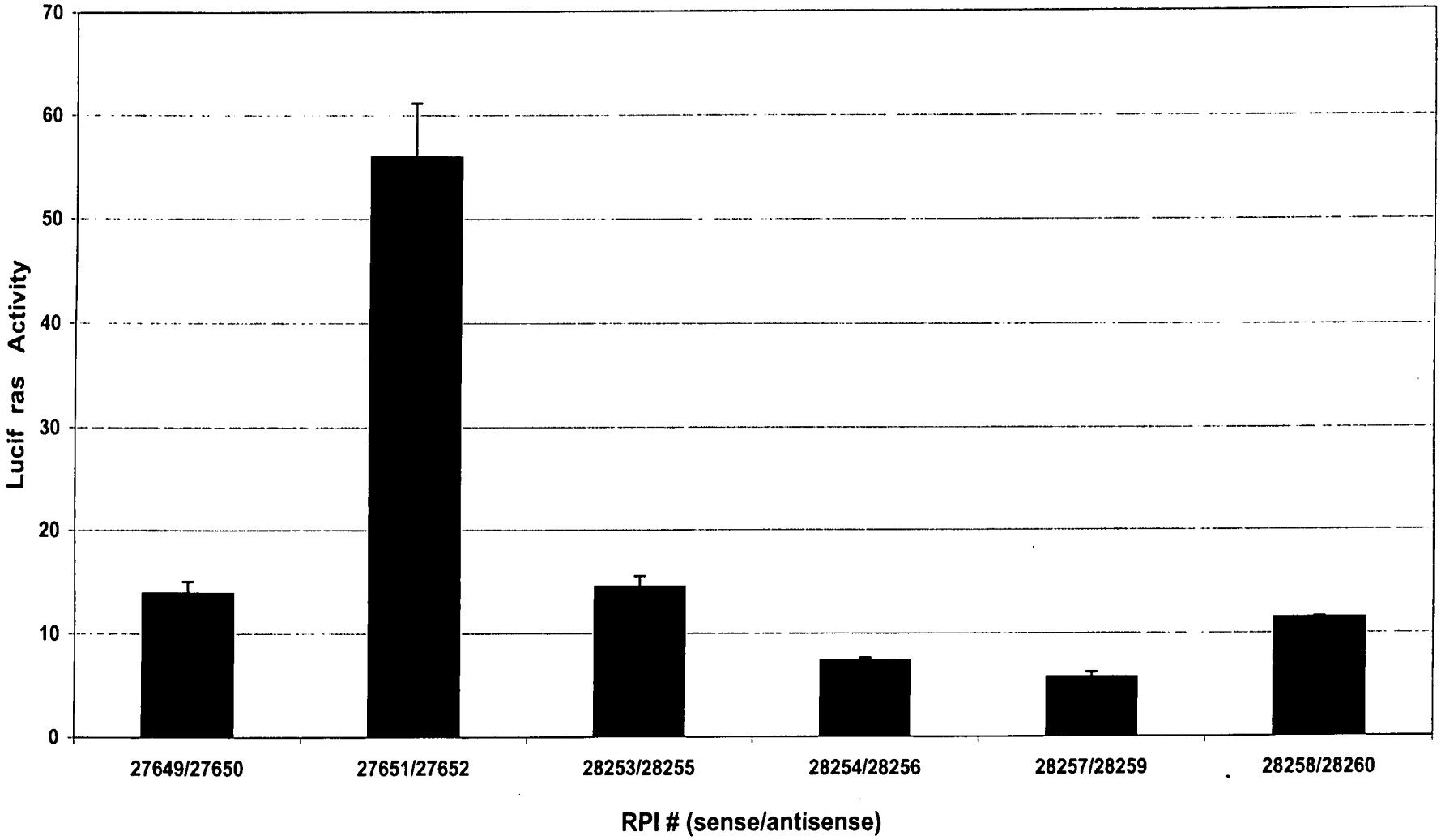

70
60
50
40
30
20
10
0
Lucif ras Activity
27649/27650
27651/27652
28253/28255
28254/28256
28257/28259
28258/28260
RPI # (sense/antisense)

Figure 6

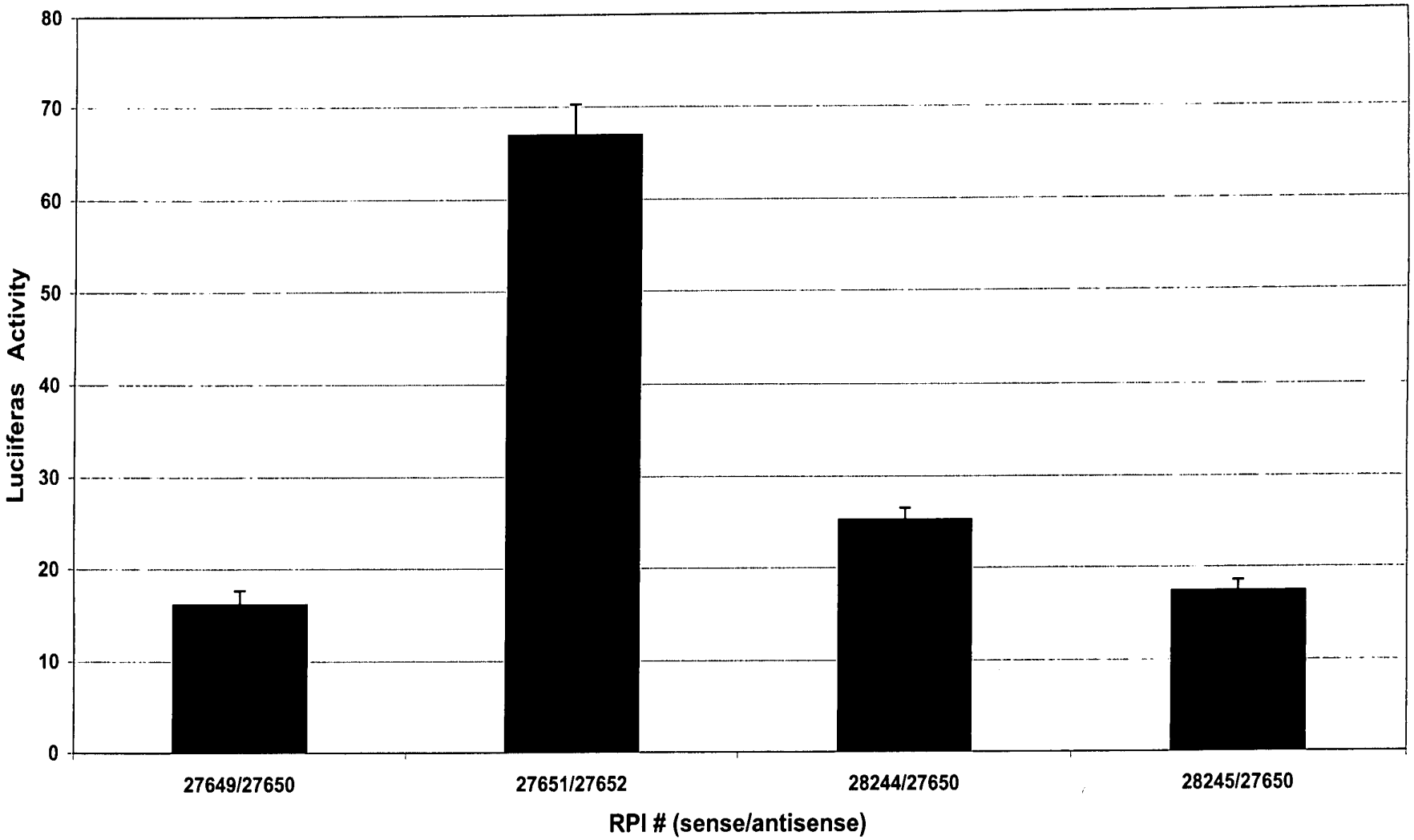

80
70
60
50
40
30
20
10
0
Luciferas Activity
27649/27650
27651/27652
28244/27650
28245/27650
RPI # (sense/antisense)

Figure 7

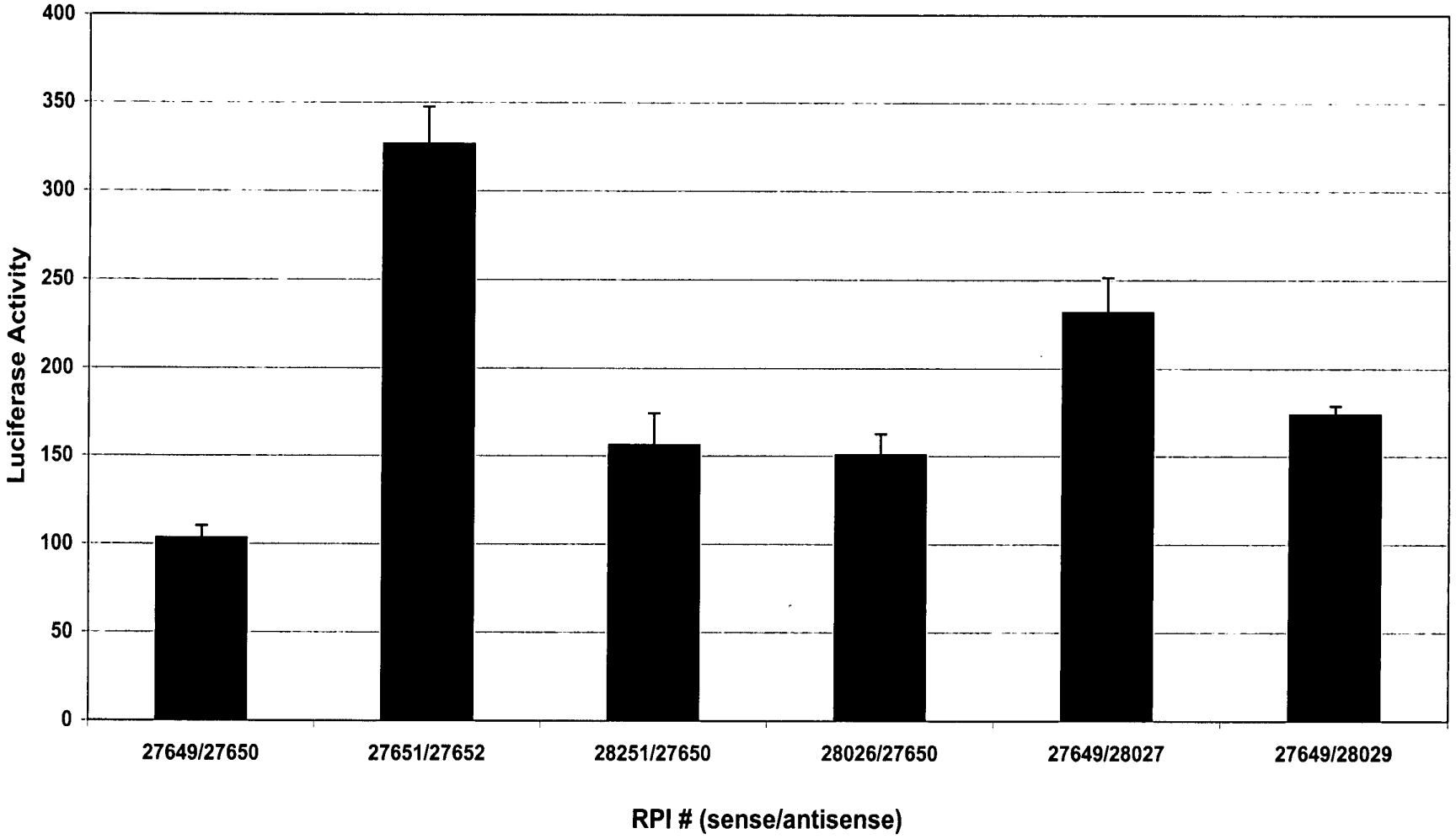

400
350
300
250
200
150
100
50
0
Luciferase Activity
27649/27650
27651/27652
28251/27650
28026/27650
27649/28027
27649/28029
RPI # (sense/antisense)

Figure 8

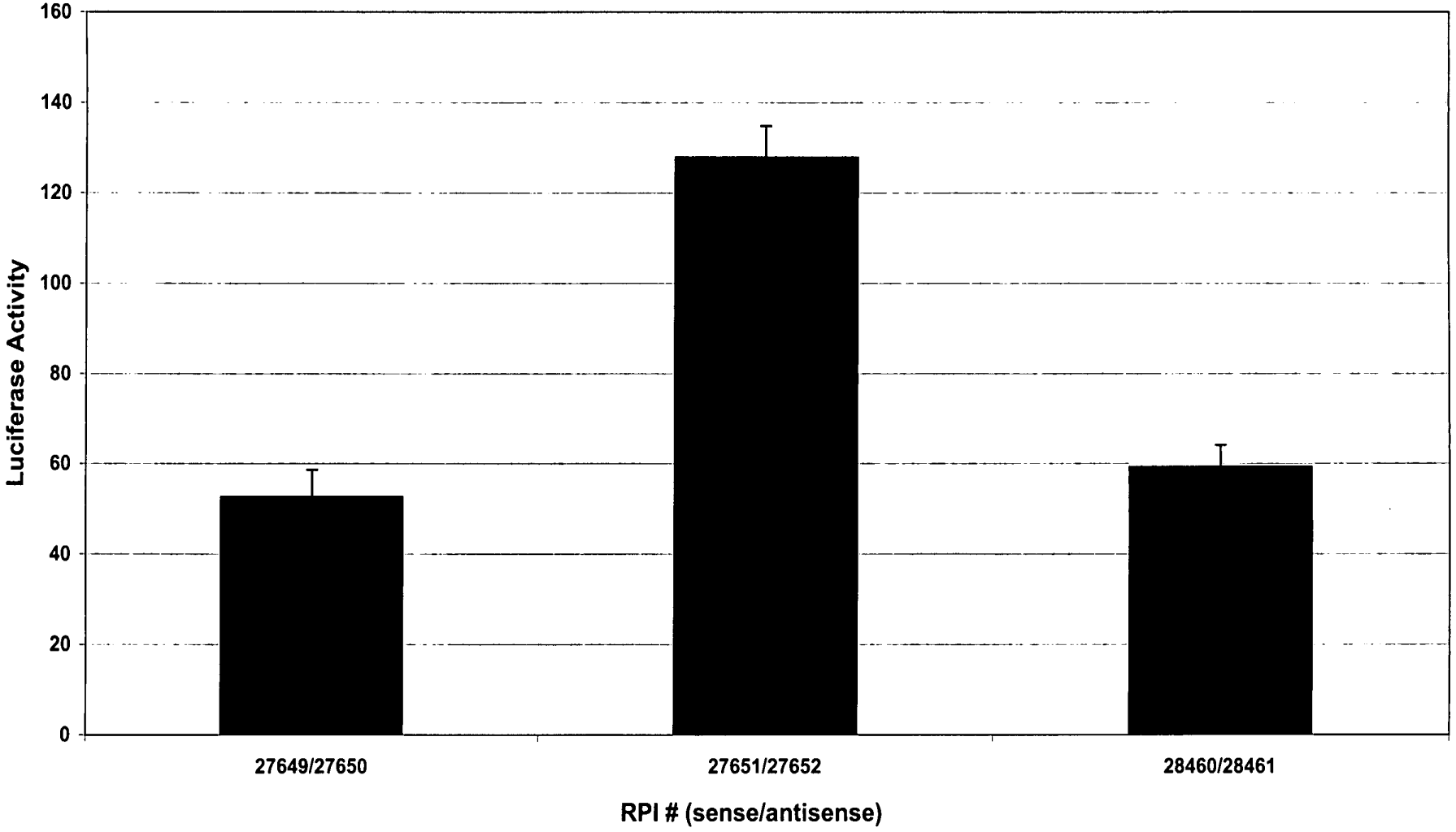

160
140
120
100
80
60
40
20
0
Luciferase Activity
27649/27650
27651/27652
28460/28461
RPI # (sense/antisense)

Figure 9

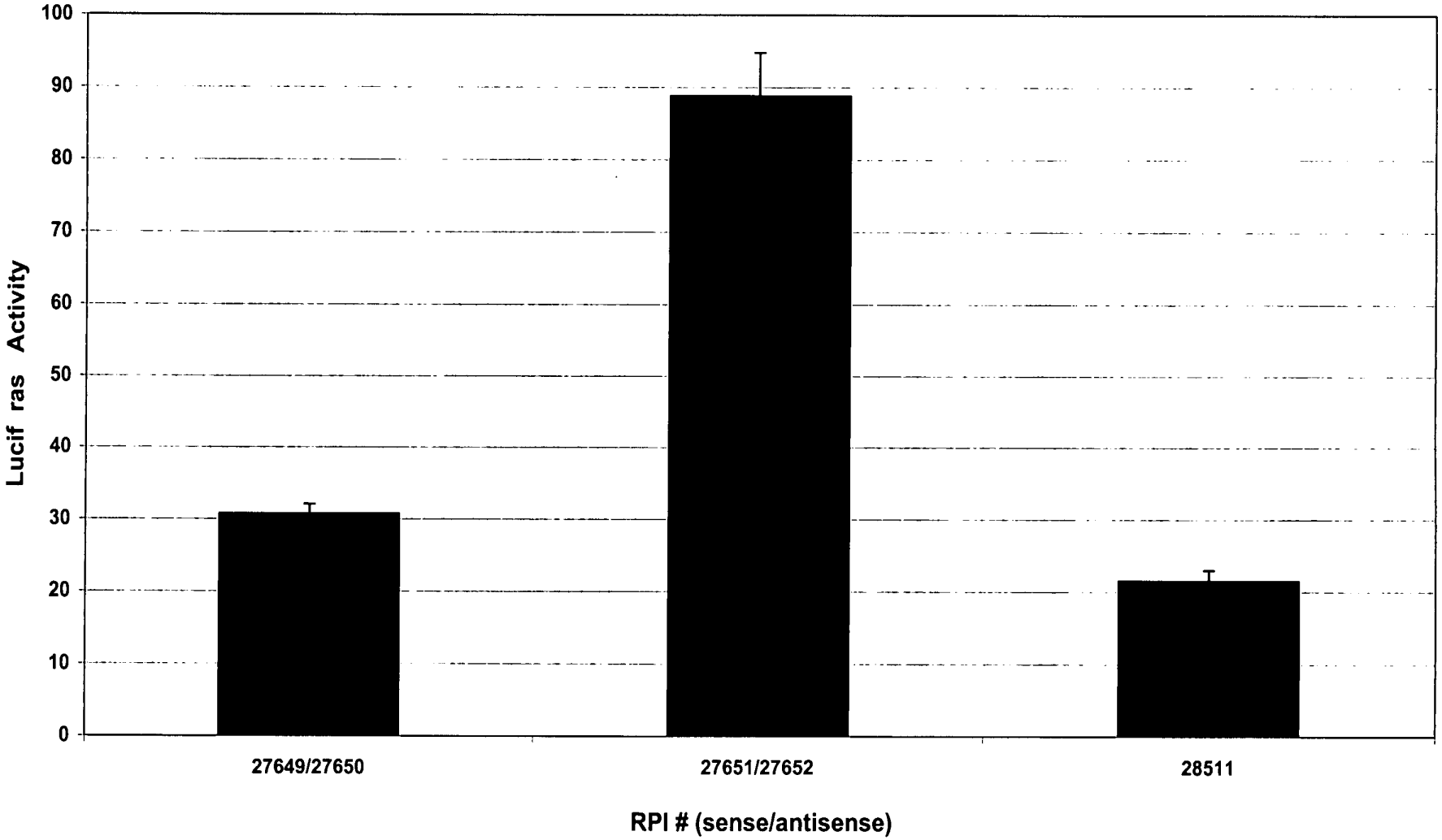

100
90
80
70
60
50
40
30
20
10
0
Lucif ras Activity
27649/27650
27651/27652
28511
RPI # (sense/antisense)

Figure 10

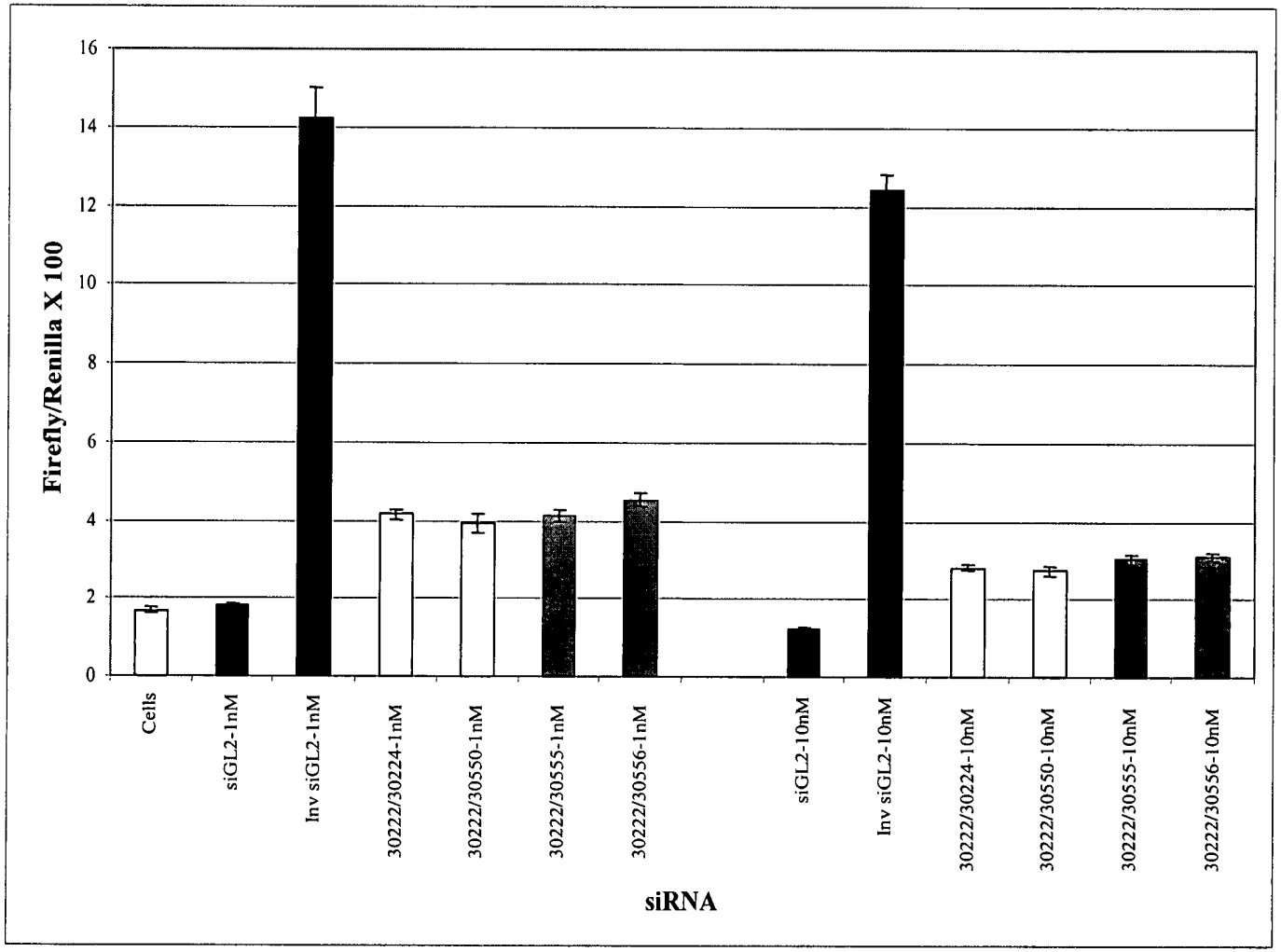

Firefly/Renilla X 100
16
14
12
10
8
6
4
2
0
Cells
siGL2-1nM
Inv siGL2-1nM
30222/30224-1nM
30222/30550-1nM
30222/30555-1nM
30222/30556-1nM
siGL2-10nM
Inv siGL2-10nM
30222/30224-10nM
30222/30550-10nM
30222/30555-10nM
30222/30556-10nM
siRNA

Figure 11

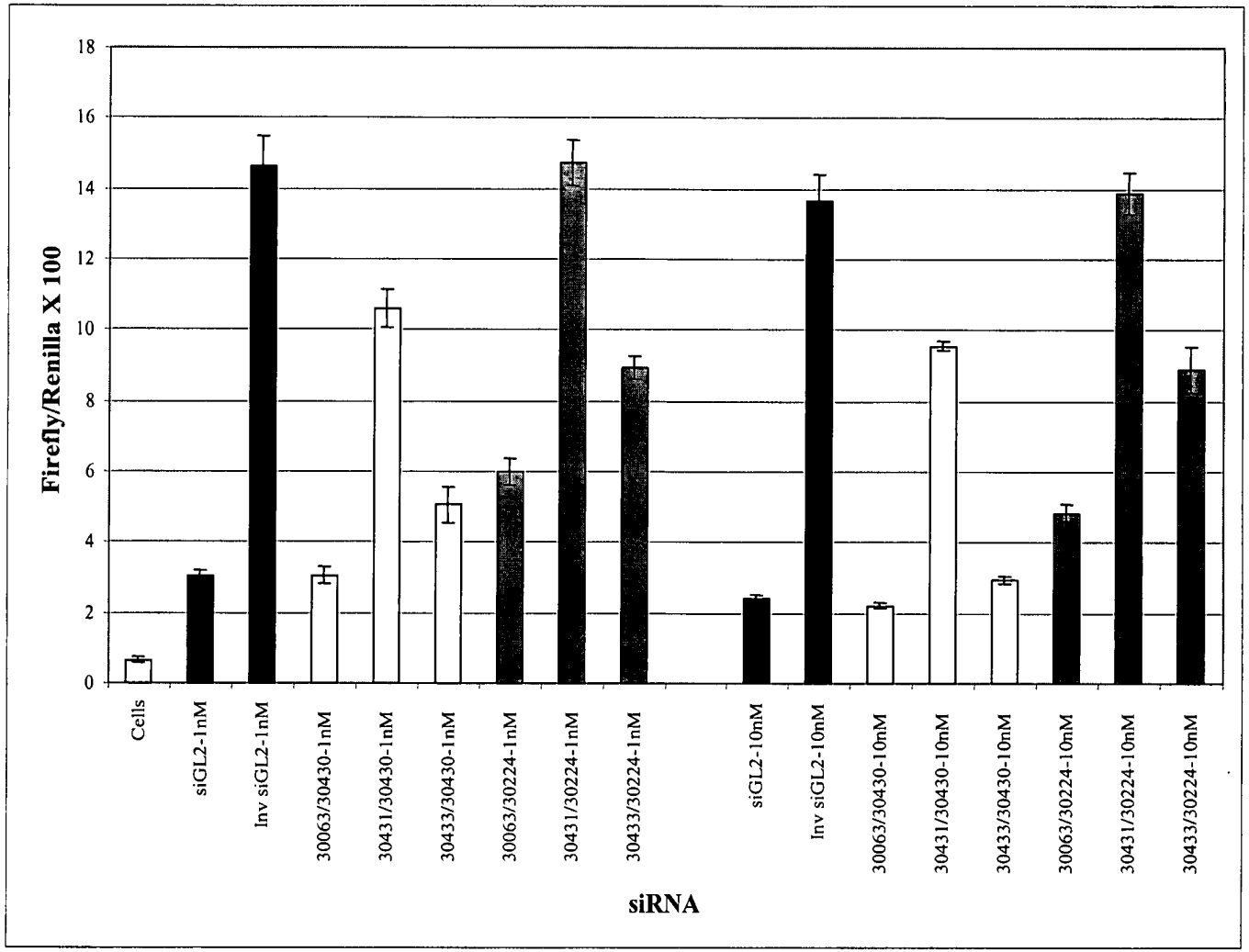

Firefly/Renilla X 100
18
16
14
12
10
8
6
4
2
0
Cells
siGL2-1nM
Inv siGL2-1nM
30063/30430-1nM
30431/30430-1nM
30433/30430-1nM
30063/30224-1nM
30431/30224-1nM
30433/30224-1nM
siGL2-10nM
Inv siGL2-10nM
30063/30430-10nM
30431/30430-10nM
30433/30430-10nM
30063/30224-10nM
30431/30224-10nM
30433/30224-10nM
siRNA

Figure 12

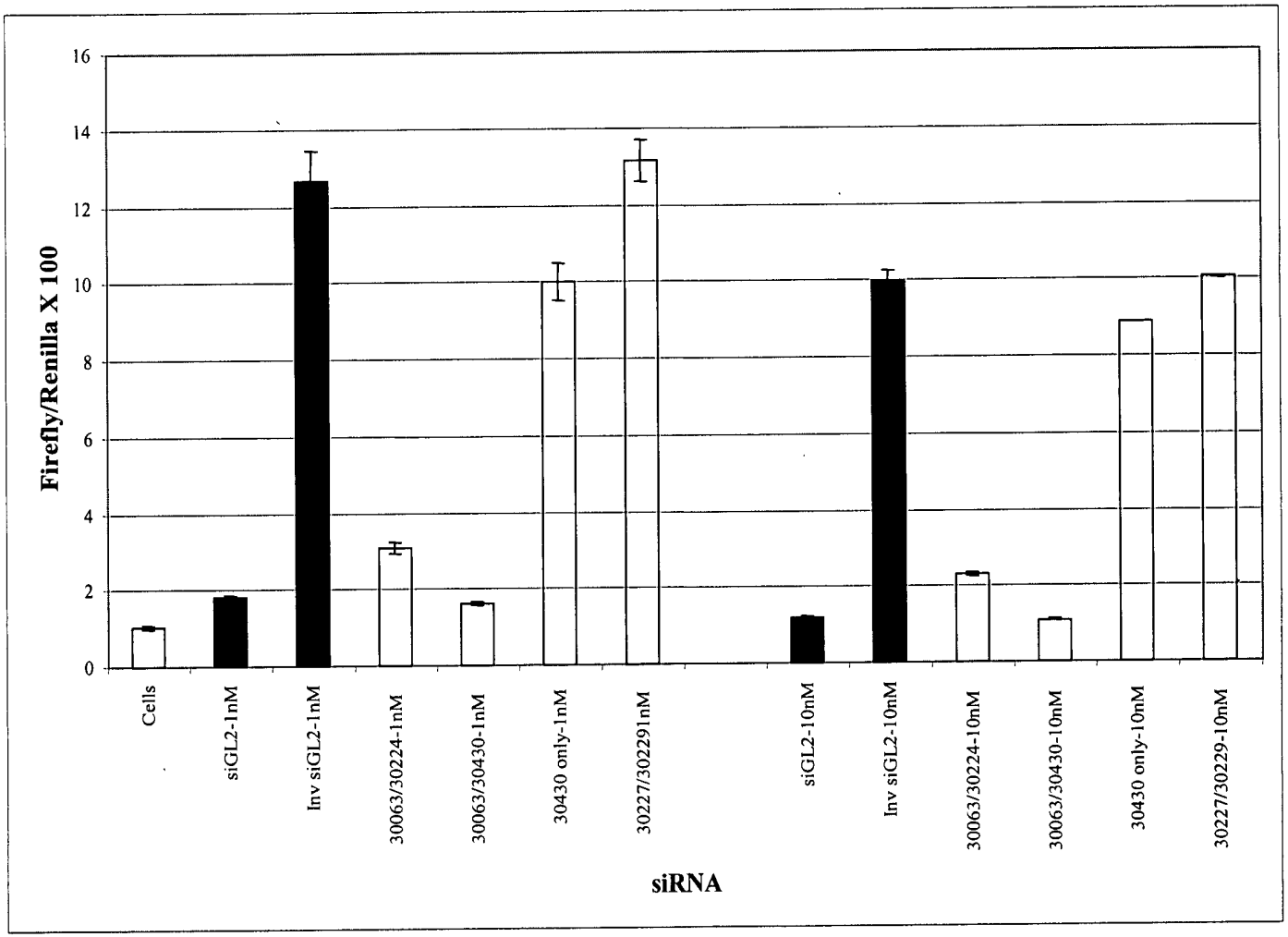

Firefly/Renilla X 100
16
14
12
10
8
6
4
2
0
Cells
siGL2-1nM
Inv siGL2-1nM
30063/30224-1nM
30063/30430-1nM
30430 only-1nM
30227/302291nM
siGL2-10nM
Inv siGL2-10nM
30063/30224-10nM
30063/30430-10nM
30430 only-10nM
30227/30229-10nM
siRNA

Figure 13

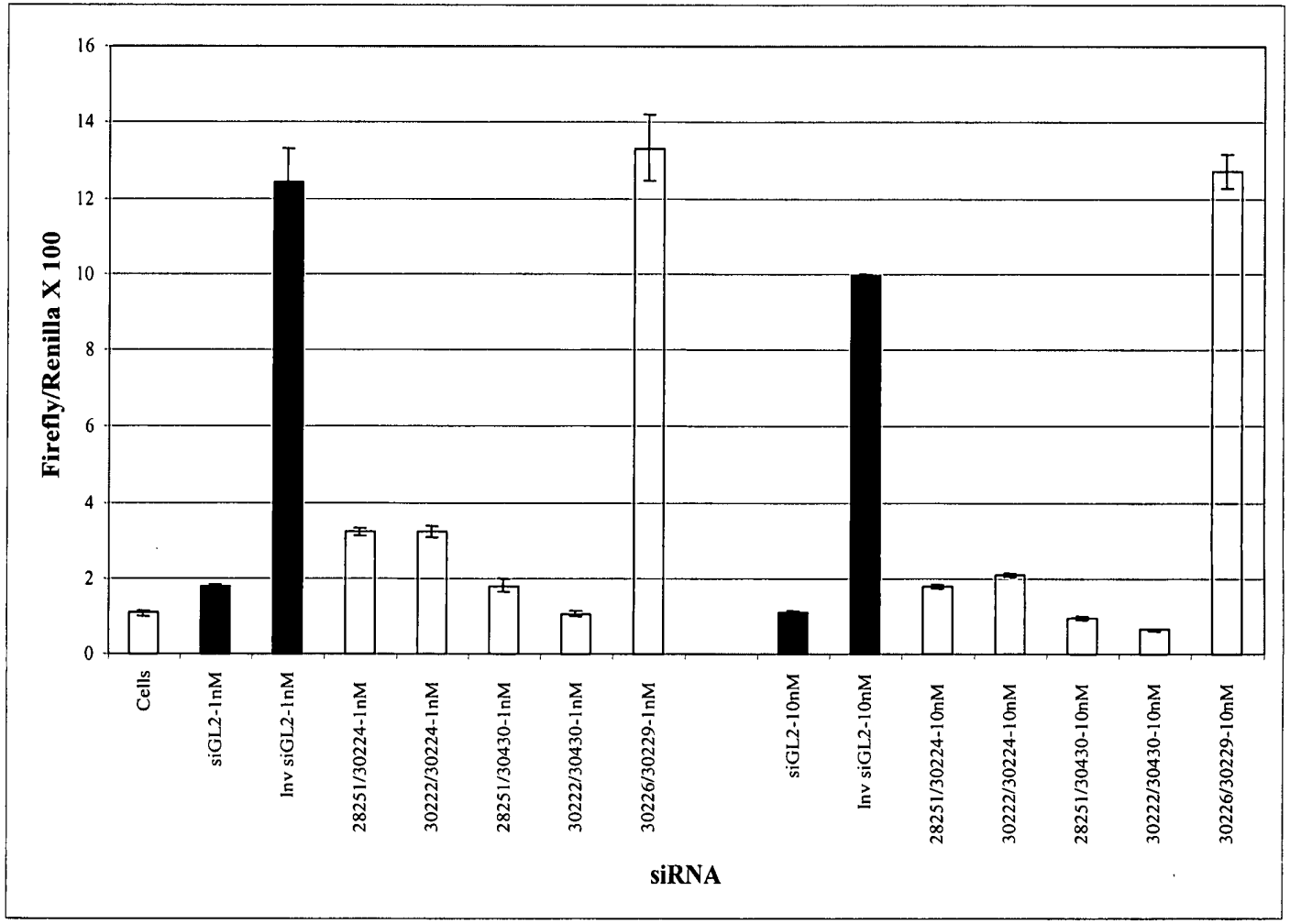

Firefly/Renilla X 100
16
14
12
10
8
6
4
2
0
Cells
siGL2-1nM
Inv siGL2-1nM
28251/30224-1nM
30222/30224-1nM
28251/30430-1nM
30222/30430-1nM
30226/30229-1nM
siGL2-10nM
Inv siGL2-10nM
28251/30224-10nM
30222/30224-10nM
28251/30430-10nM
30222/30430-10nM
30226/30229-10nM
siRNA

Figure 14

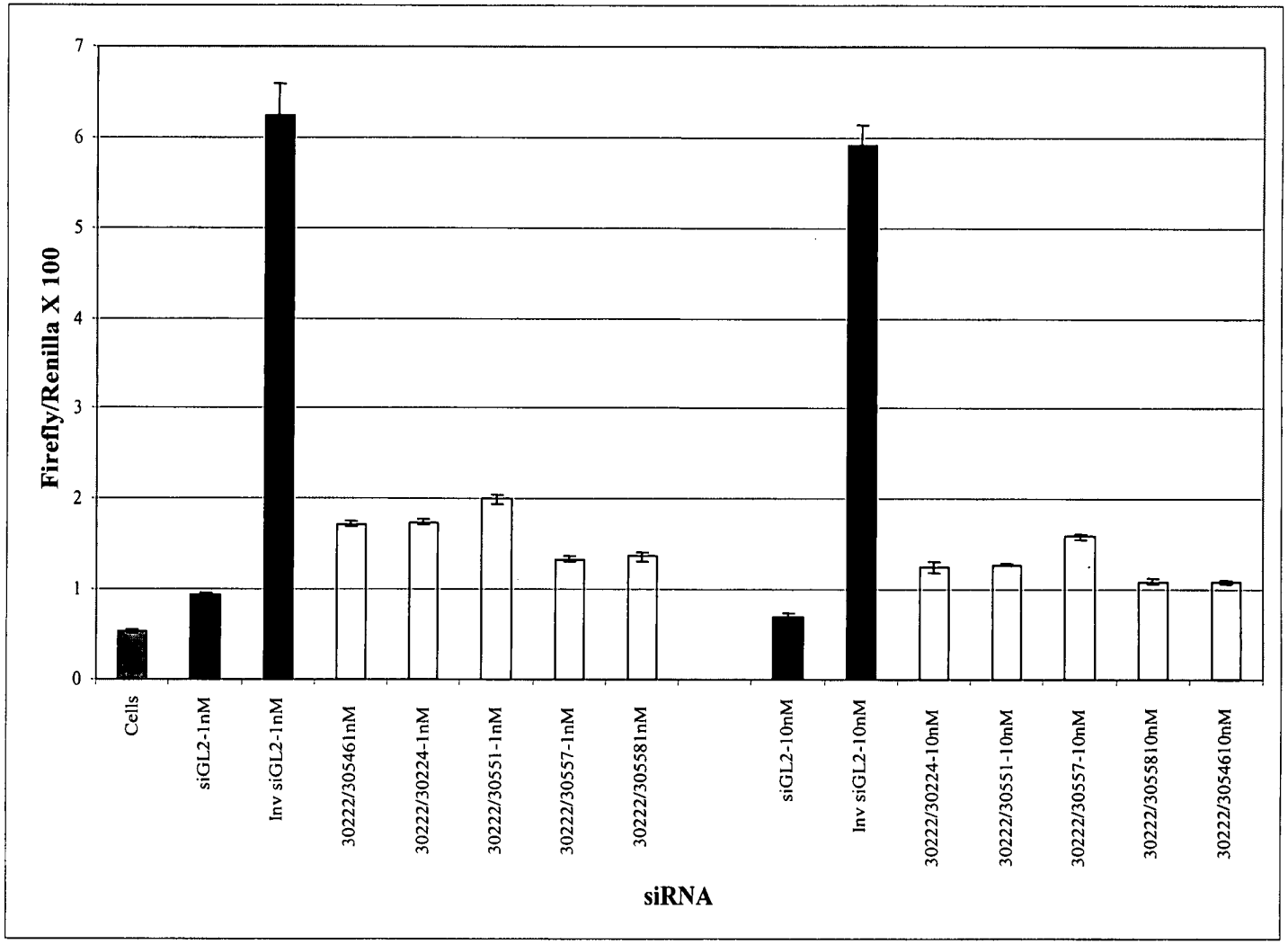

Firefly/Renilla X 100
0
1
2
3
4
5
6
7
Cells
siGL2-1nM
Inv siGL2-1nM
30222/30546 1nM
30222/30224-1nM
30222/30551-1nM
30222/30557-1nM
30222/30558 1nM
siGL2-10nM
Inv siGL2-10nM
30222/30224-10nM
30222/30551-10nM
30222/30557-10nM
30222/30558 10nM
30222/30546 10nM
siRNA

Figure 15

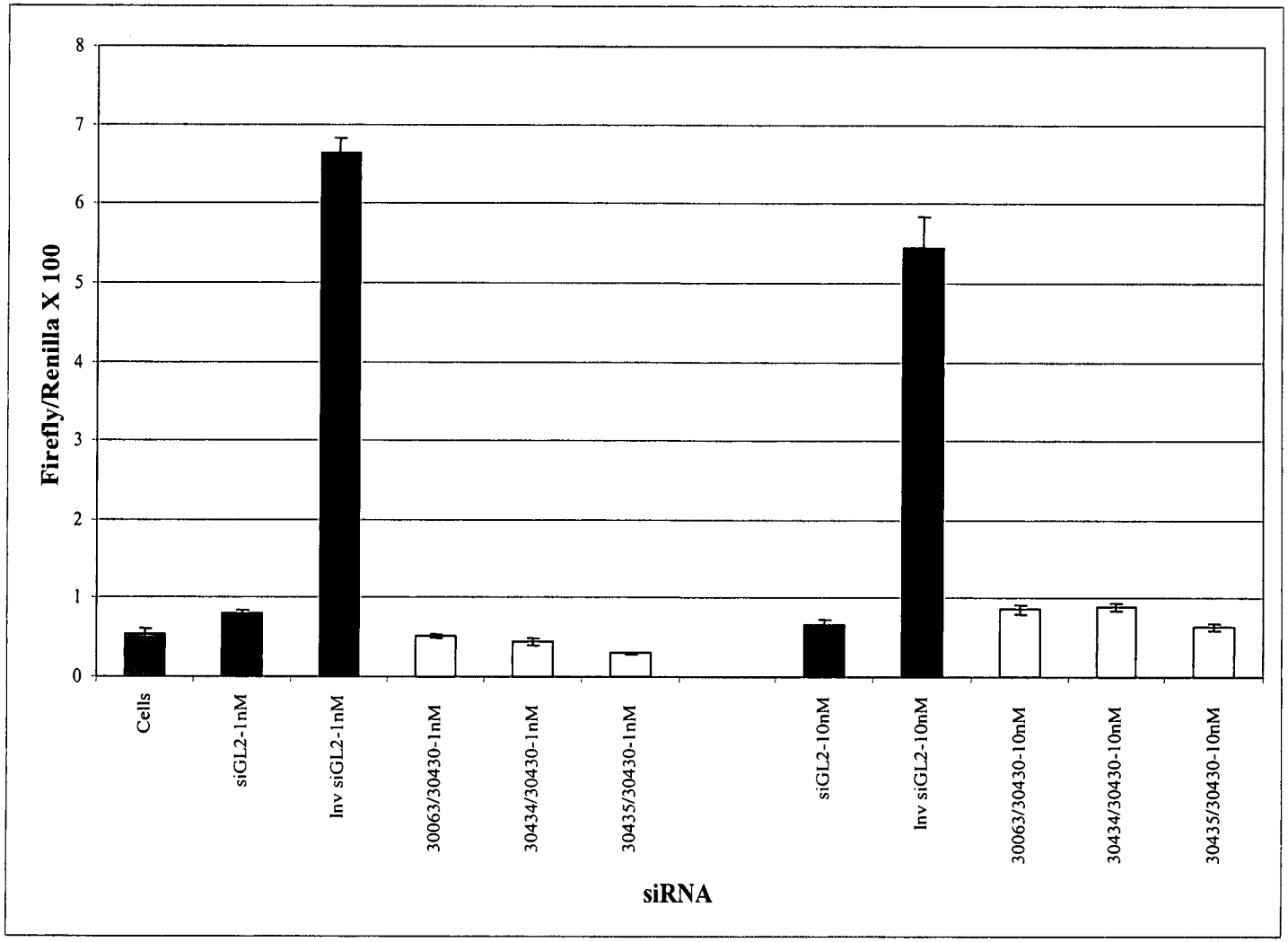

Firefly/Renilla X 100
8
7
6
5
4
3
2
1
0
Cells
siGL2-1nM
Inv siGL2-1nM
30063/30430-1nM
30434/30430-1nM
30435/30430-1nM
siGL2-10nM
Inv siGL2-10nM
30063/30430-10nM
30434/30430-10nM
30435/30430-10nM
siRNA

Figure 16

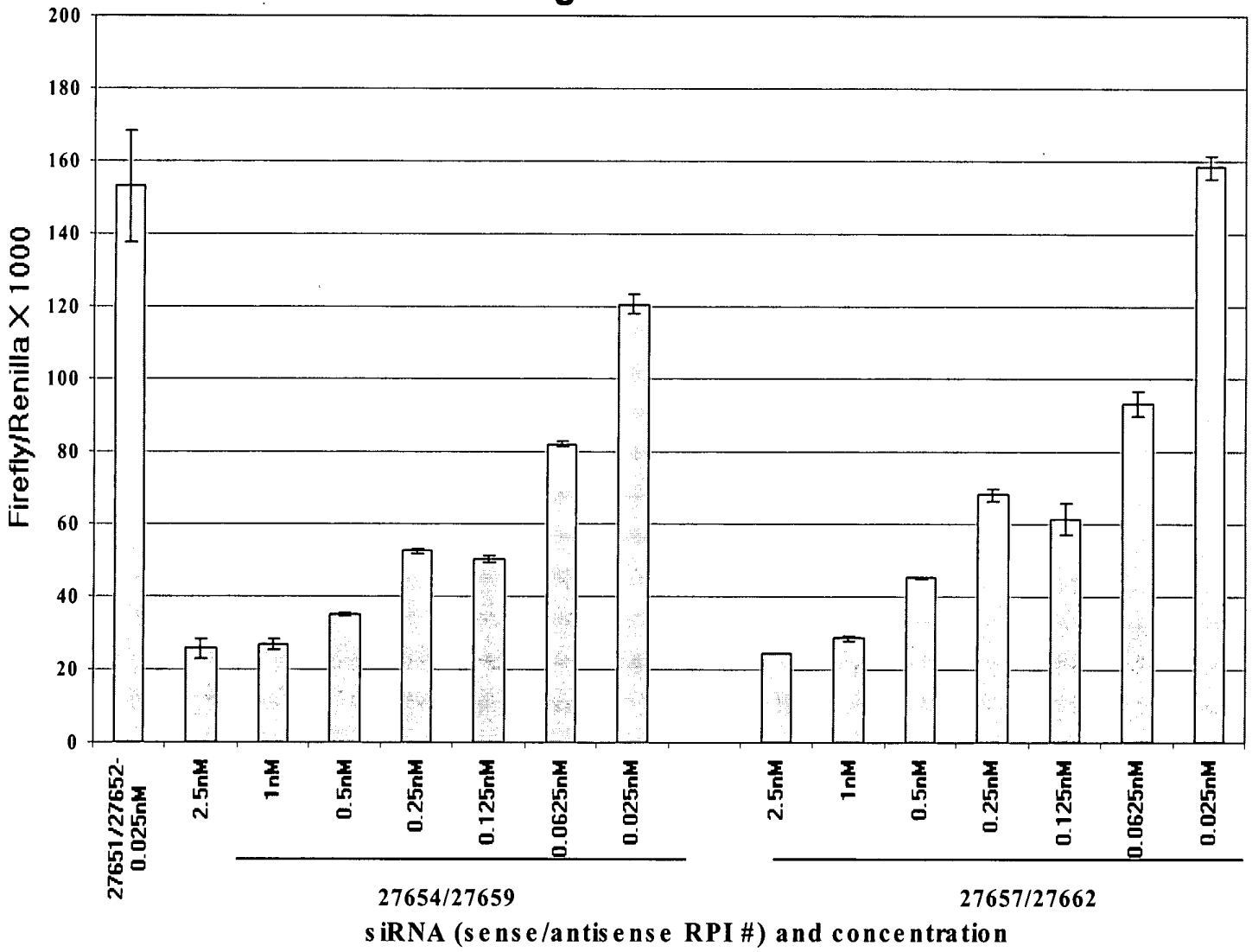

Firefly/Renilla X 1000
200
180
160
140
120
100
80
60
40
20
0
27651/27652-0.025nM
2.5nM
1nM
0.5nM
0.25nM
0.125nM
0.0625nM
0.025nM
2.5nM
1nM
0.5nM
0.25nM
0.125nM
0.0625nM
0.025nM
27654/27659
27657/27662
siRNA (sense/antisense RPI #) and concentration

Figure 17

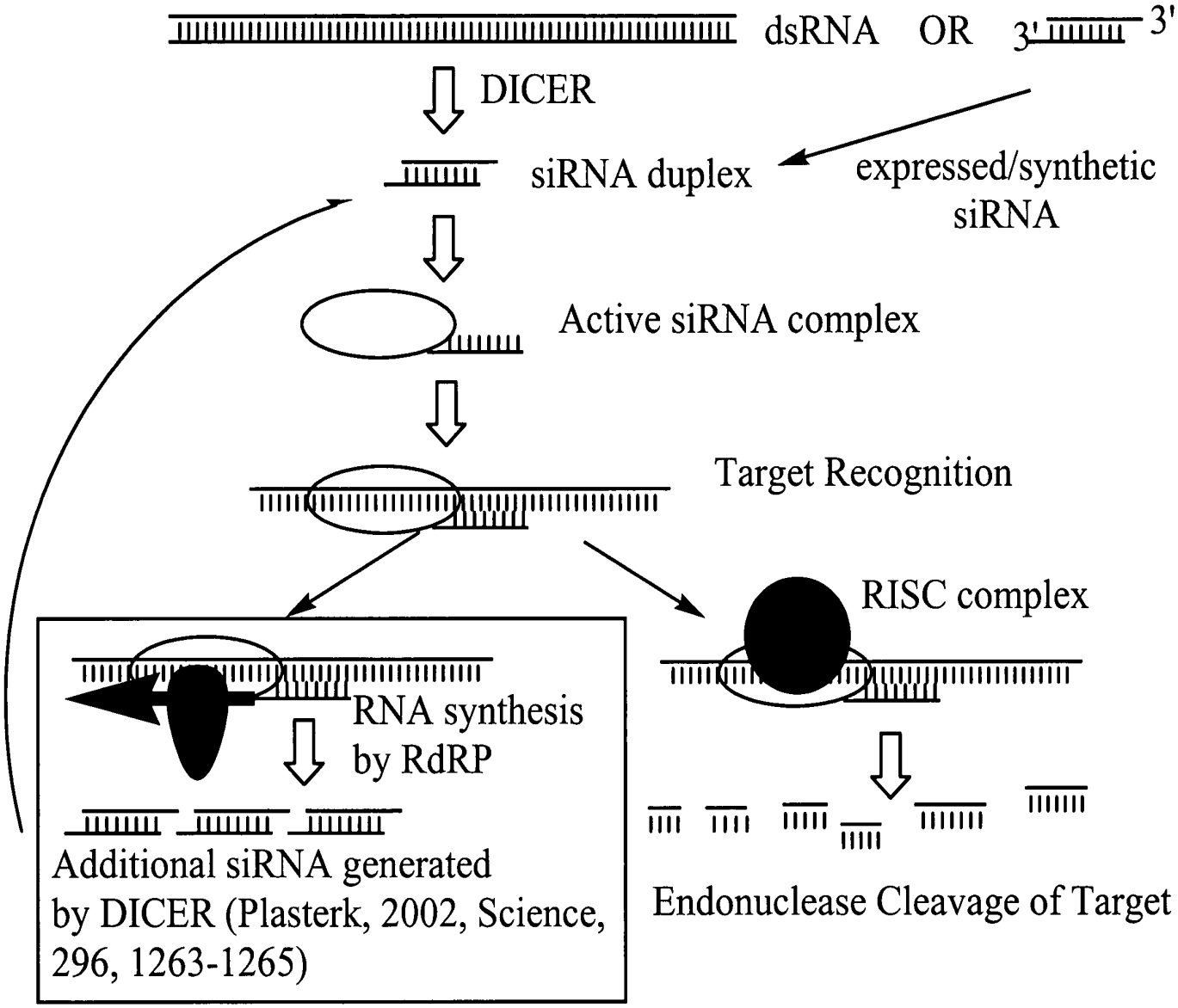
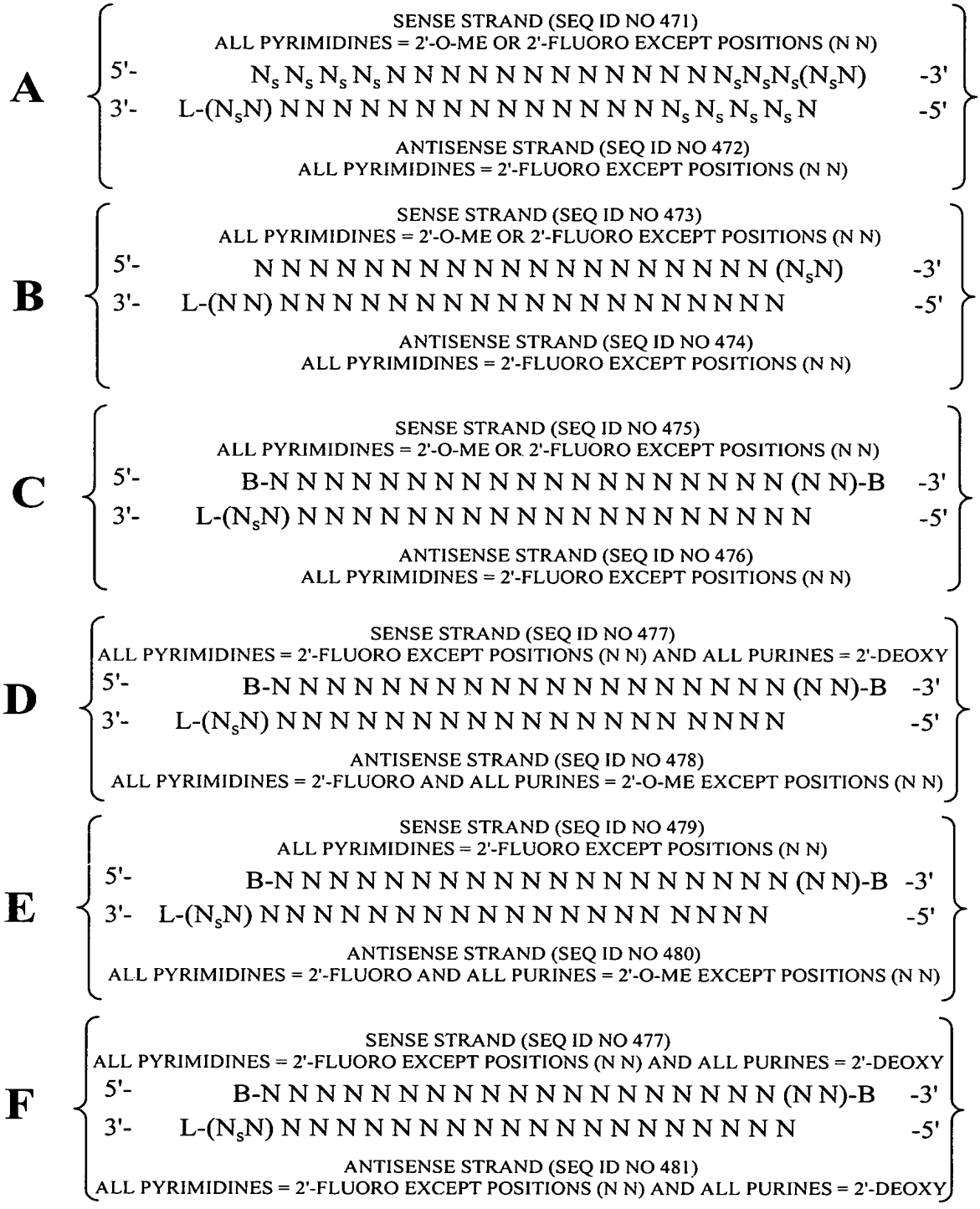

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)
Endonuclease Cleavage of Target

Figure 18



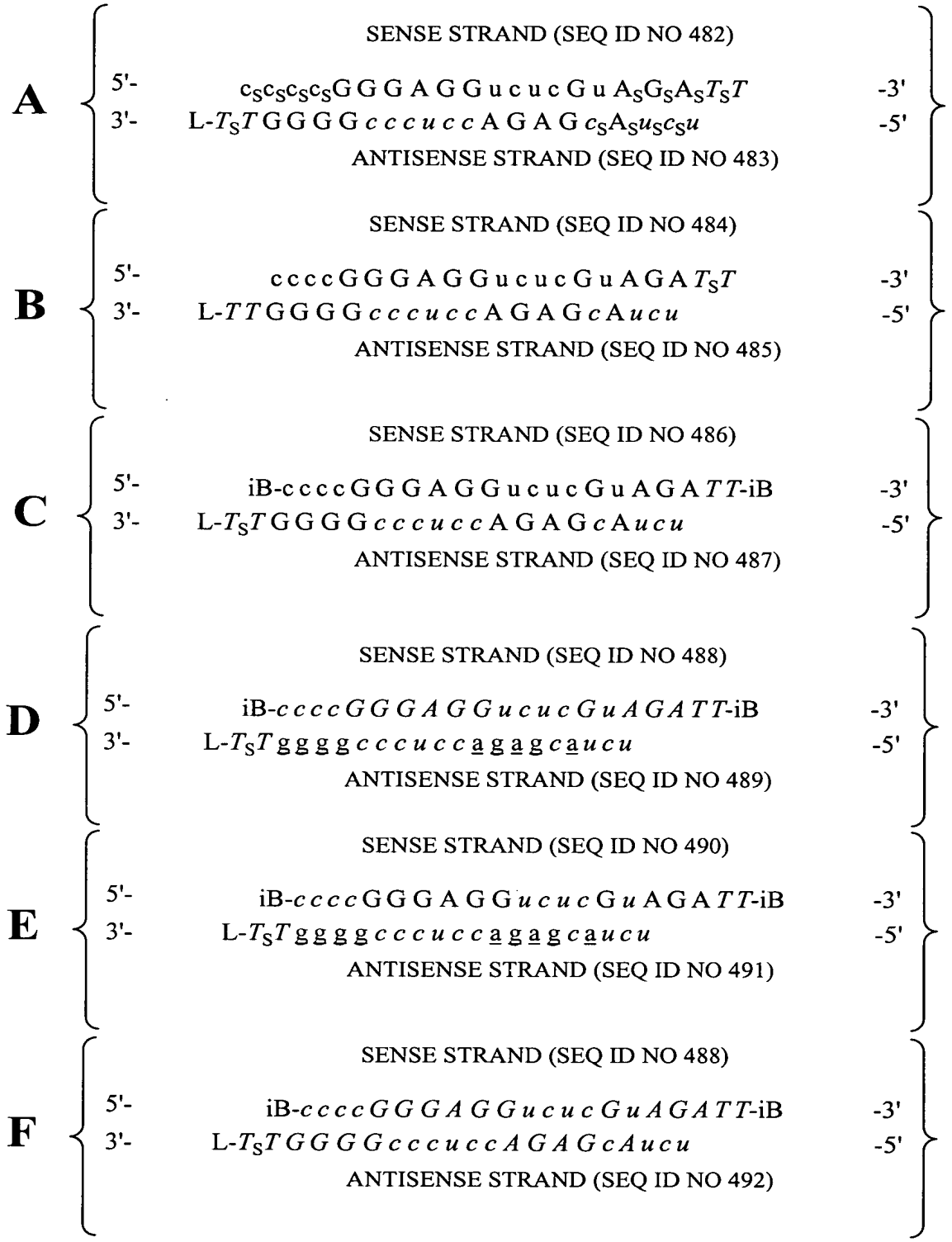
POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES

B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT

L = GLYCERYL MOIETY THAT IS OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

Figure 19



lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro
italic lower case = 2'-deoxy-2'-fluoro
underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY
B = INVERTED DEOXYABASIC
L = GLYCERYL MOIETY OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

Figure 20

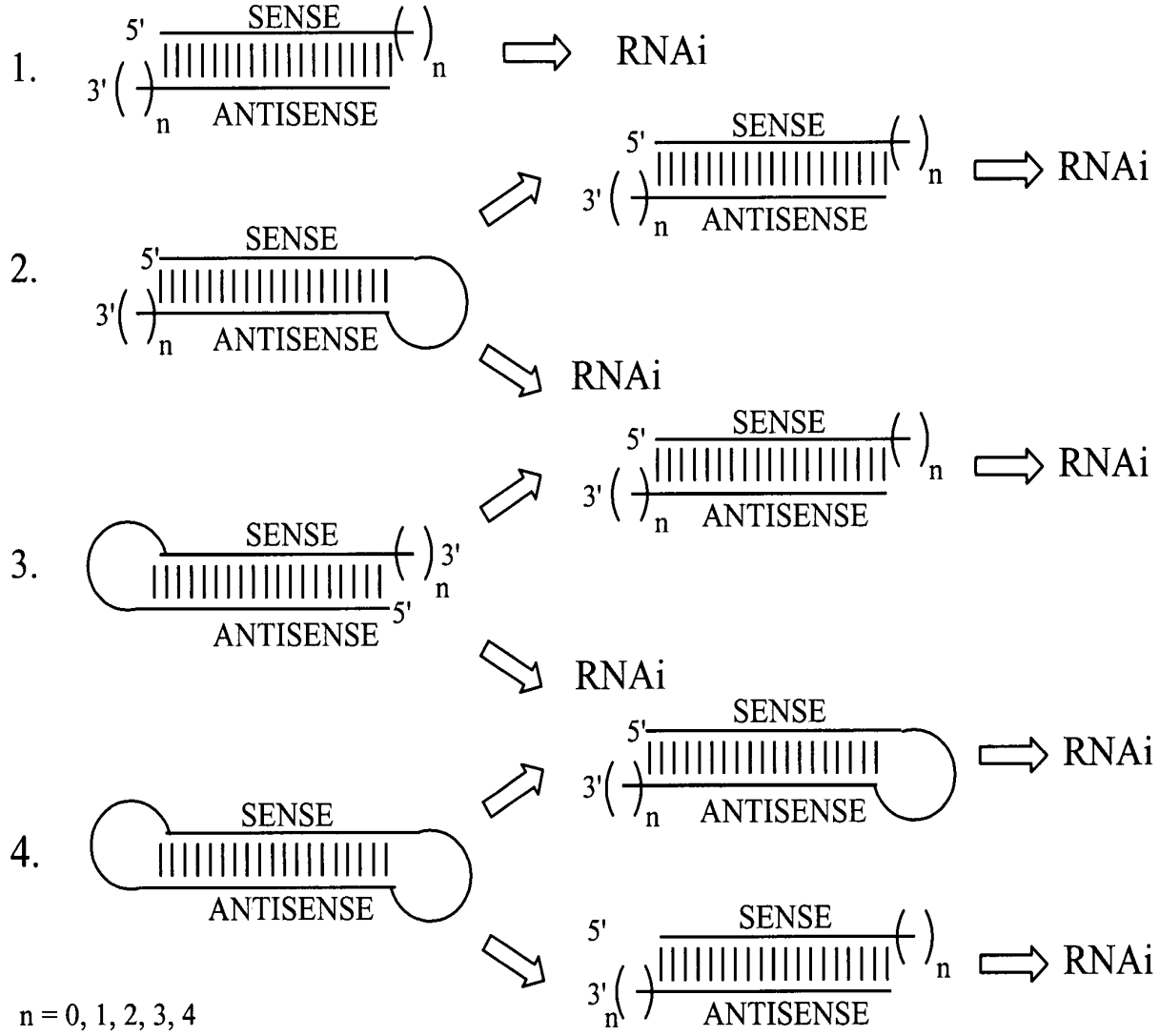

1.
5'
SENSE
3'
n
ANTISENSE
n
RNAi
2.
5'
SENSE
3'
n
ANTISENSE
RNAi
5'
SENSE
3'
n
ANTISENSE
n
RNAi
3.
SENSE
3'
n
ANTISENSE
5'
RNAi
5'
SENSE
3'
n
ANTISENSE
n
RNAi
4.
SENSE
ANTISENSE
5'
SENSE
3'
n
ANTISENSE
RNAi
5'
SENSE
3'
n
ANTISENSE
n
RNAi
n = 0, 1, 2, 3, 4

Figure 21: Target site Selection using siRNA

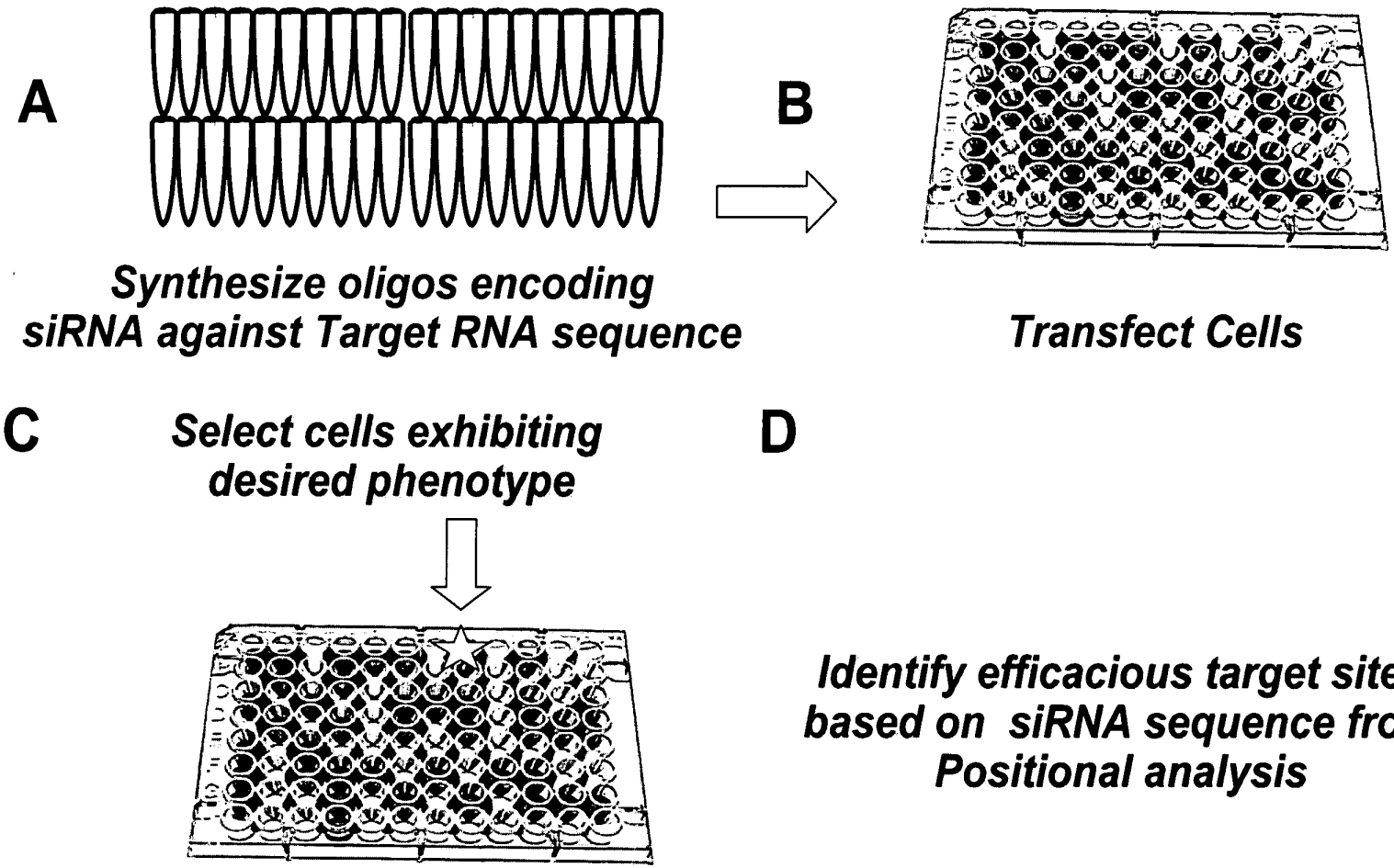
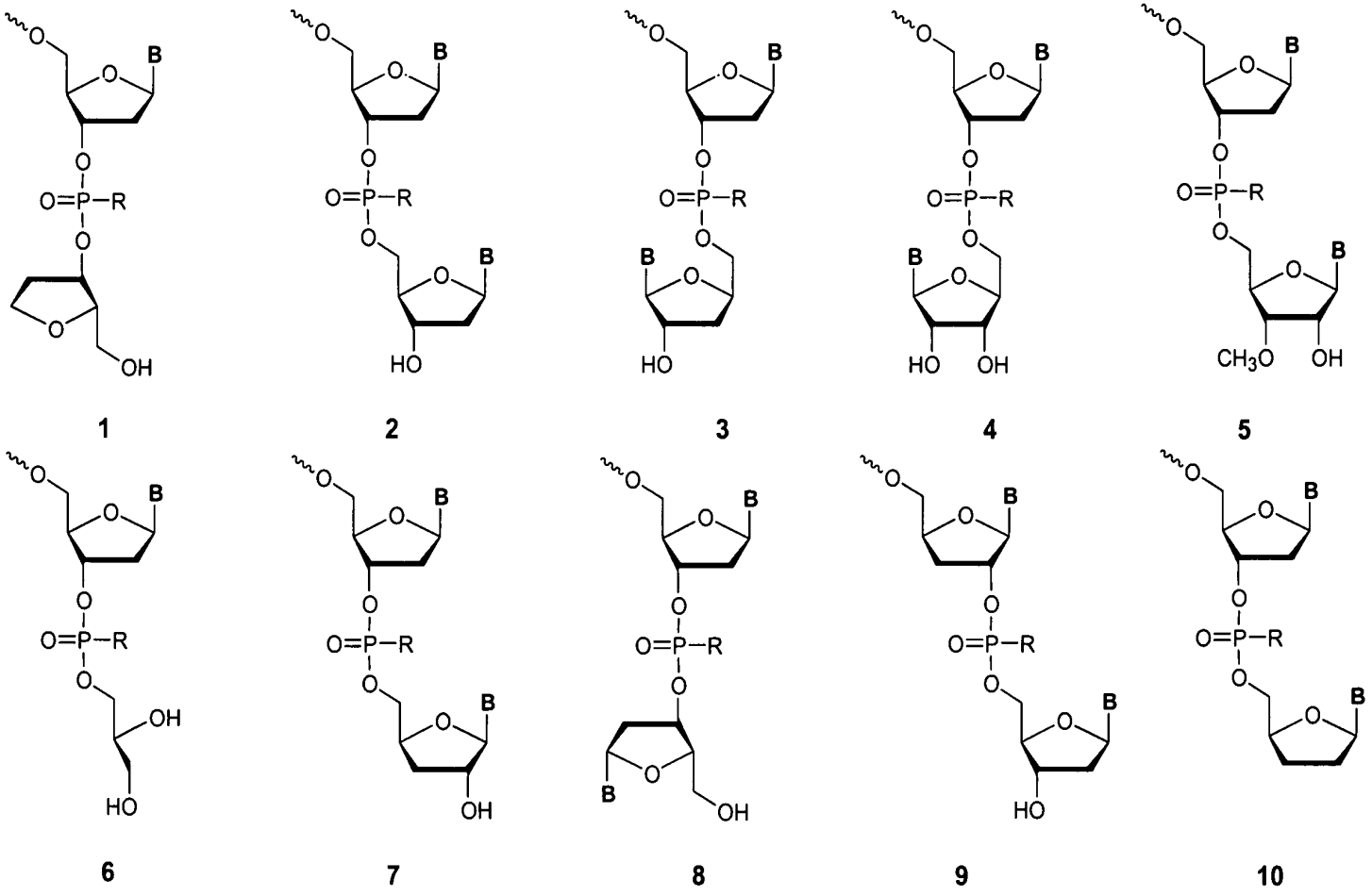


Figure 22



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 23: Inhibition of VEGF-Induced Angiogenesis by siRNAs

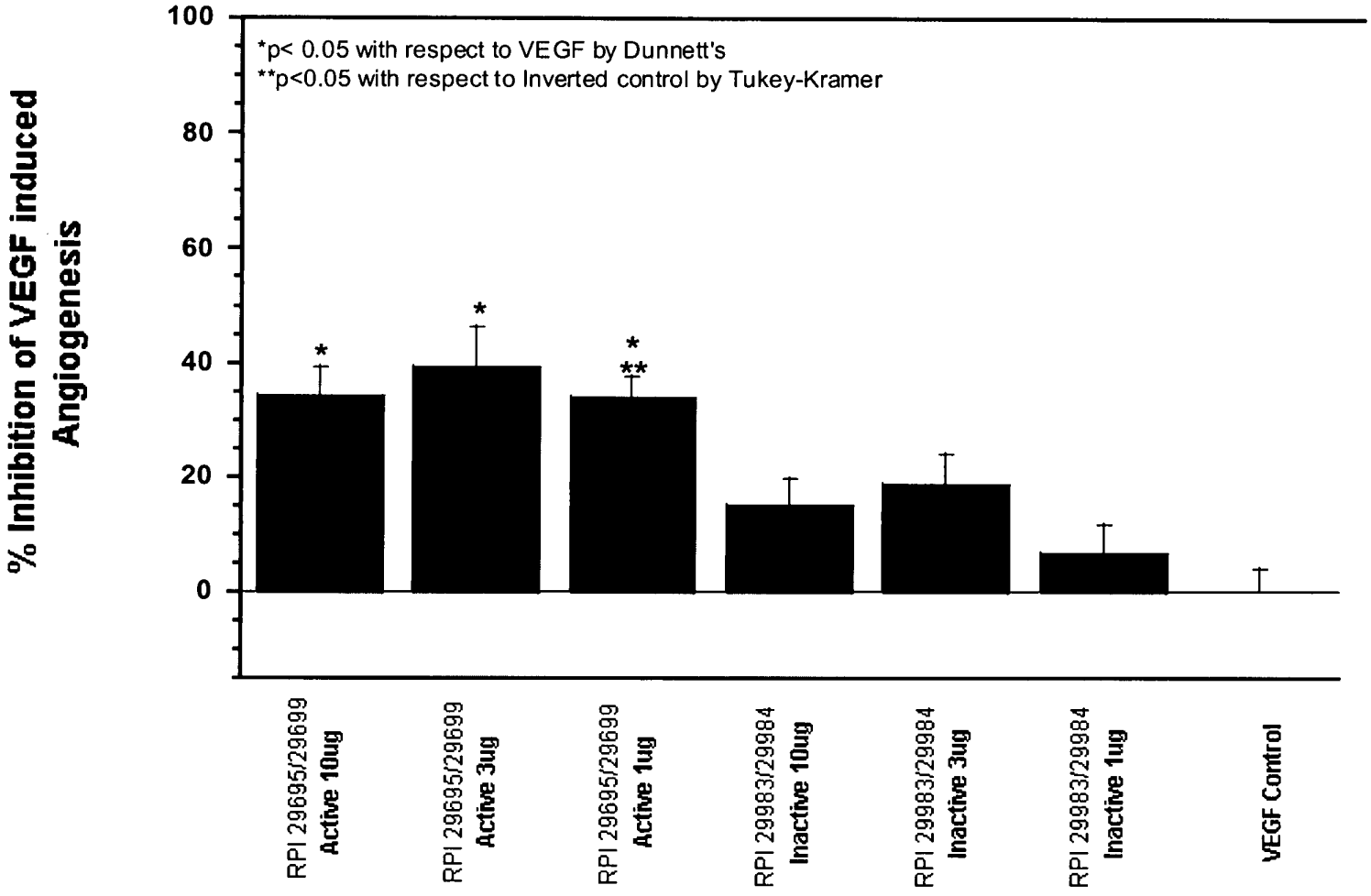


Figure 24: Stab4/5 siNA Targeted to HBV: HBsAg Levels in Hep G2 Cells

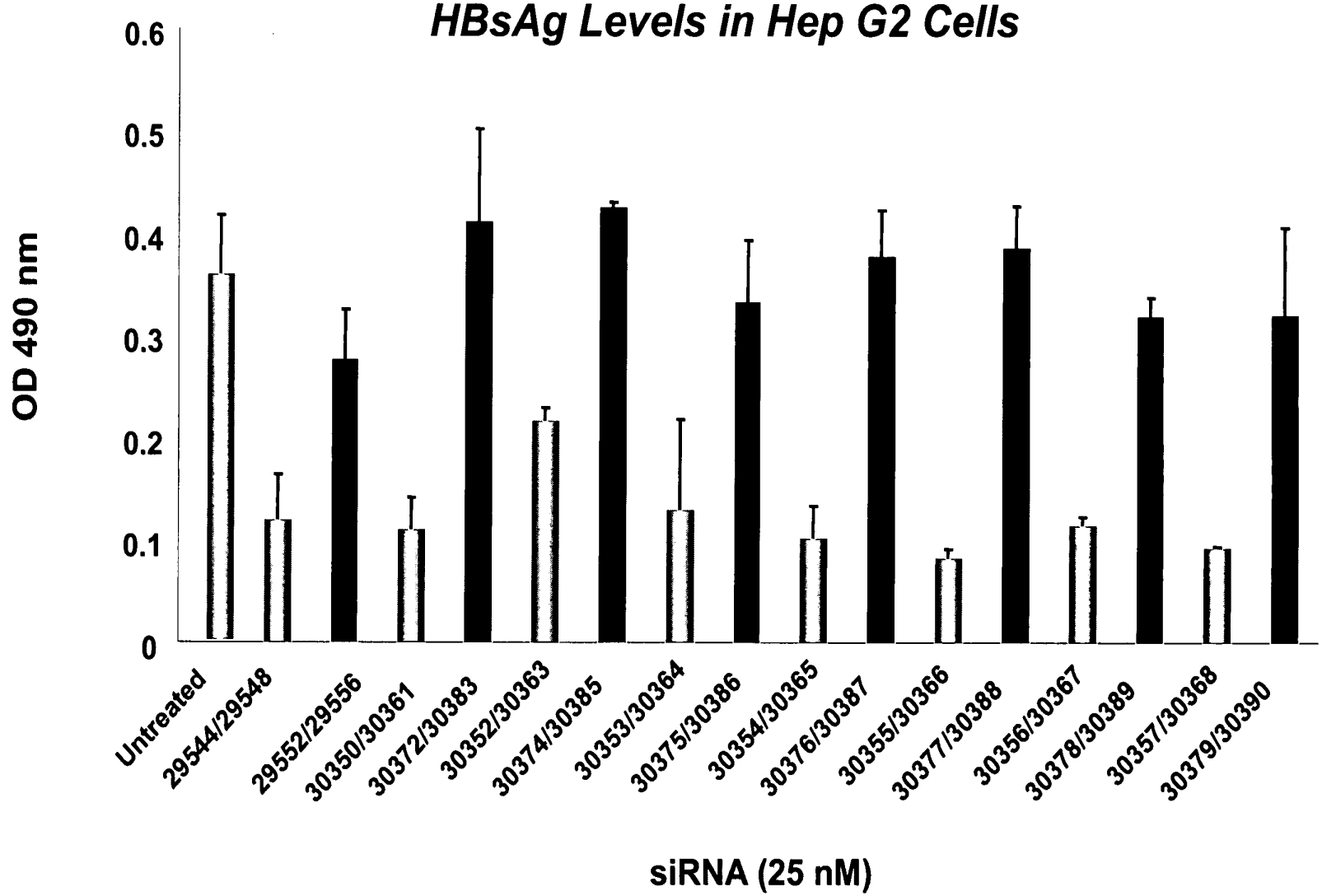


Figure 25: Dose Response with Stab4/5 siRNAs Targeted to HBV Sites 262 & 1580

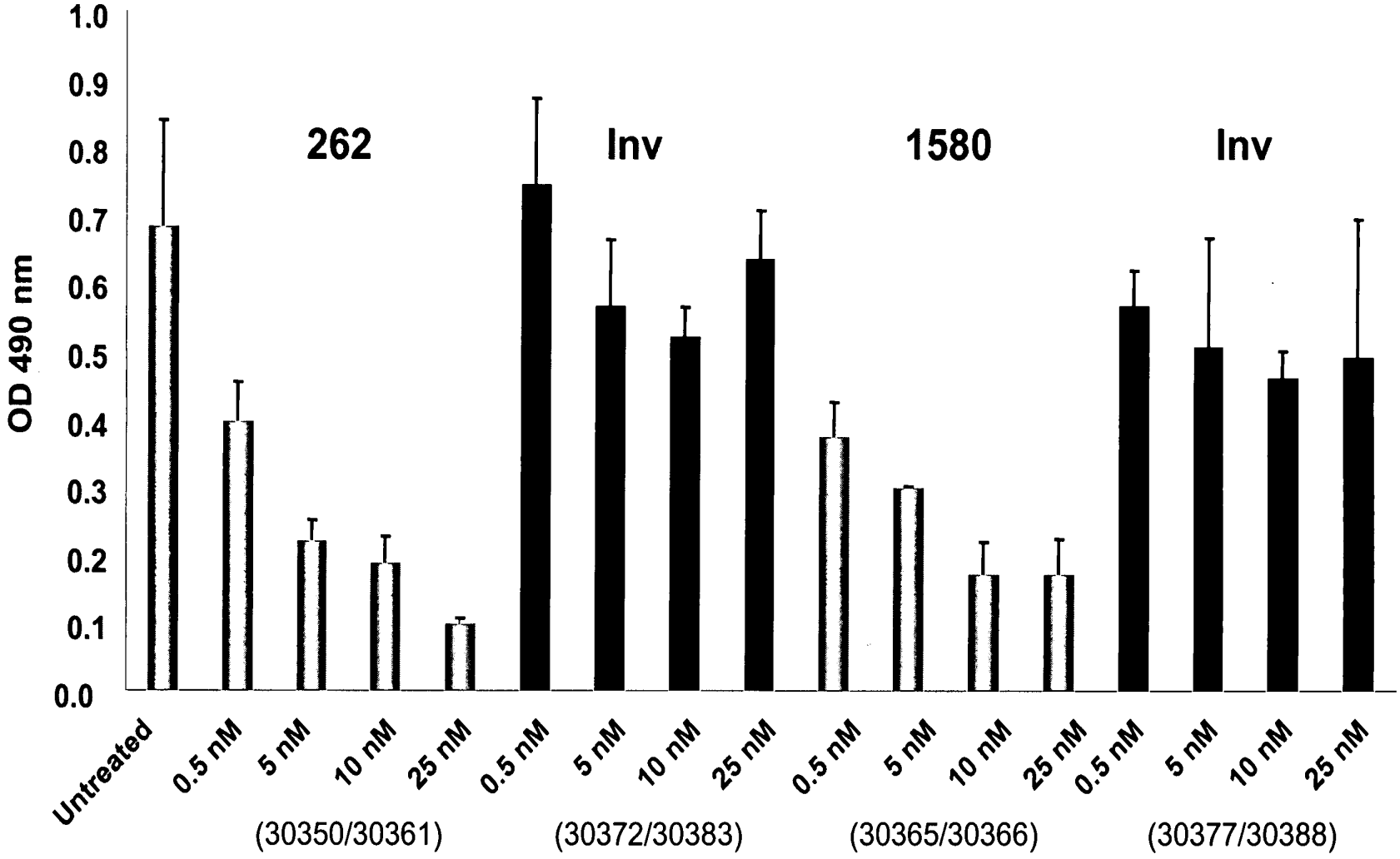


Figure 26: Comparison of Stab7/8 and Stab 7/11 siRNAs Targeted to HBV RNA Site 1580

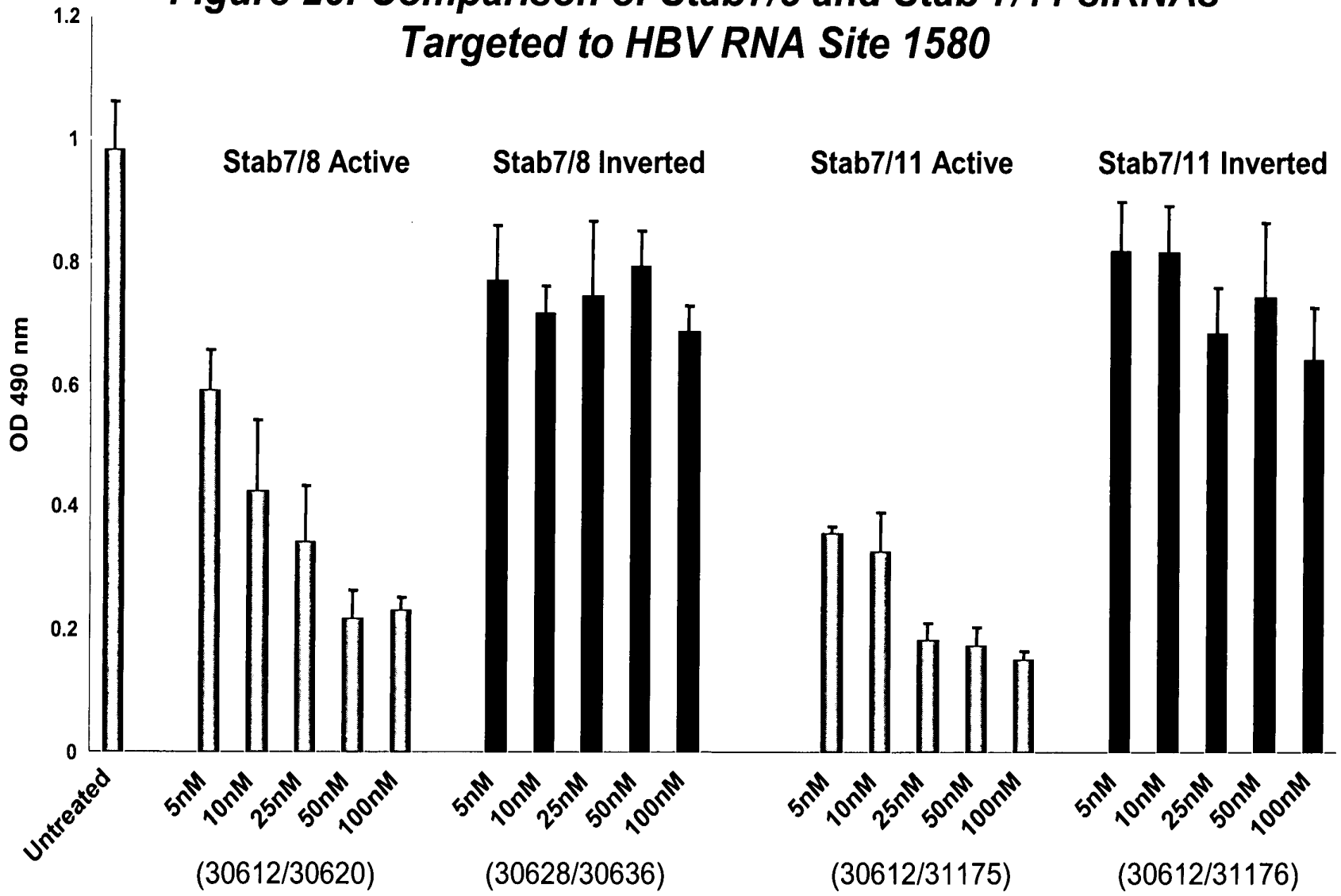


Figure 27: Modification Strategy

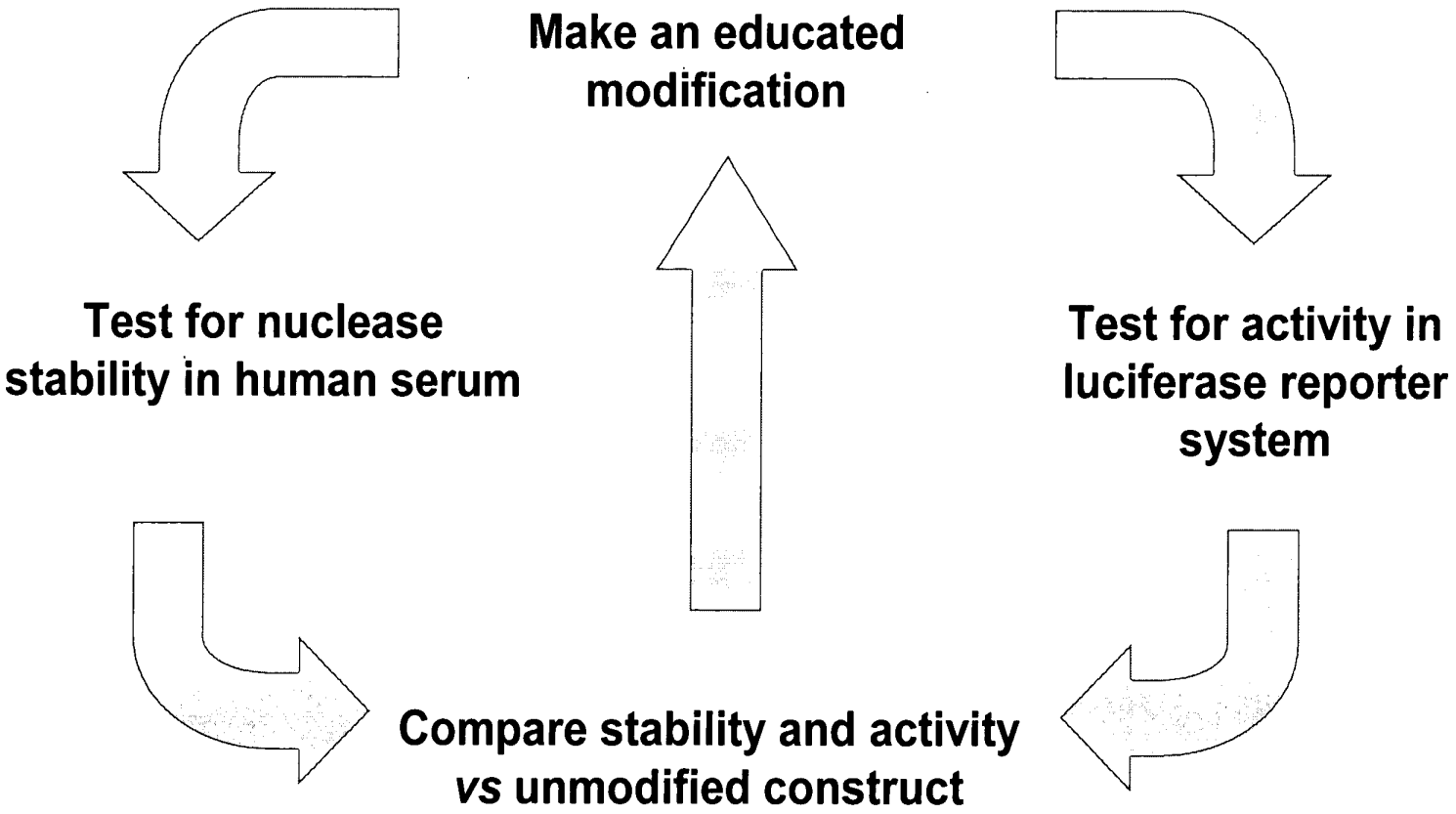


Figure 28: Duration of siRNA Effect
All-Ribo vs. Stab4/5 HBV Site 1580: HBsAg Levels

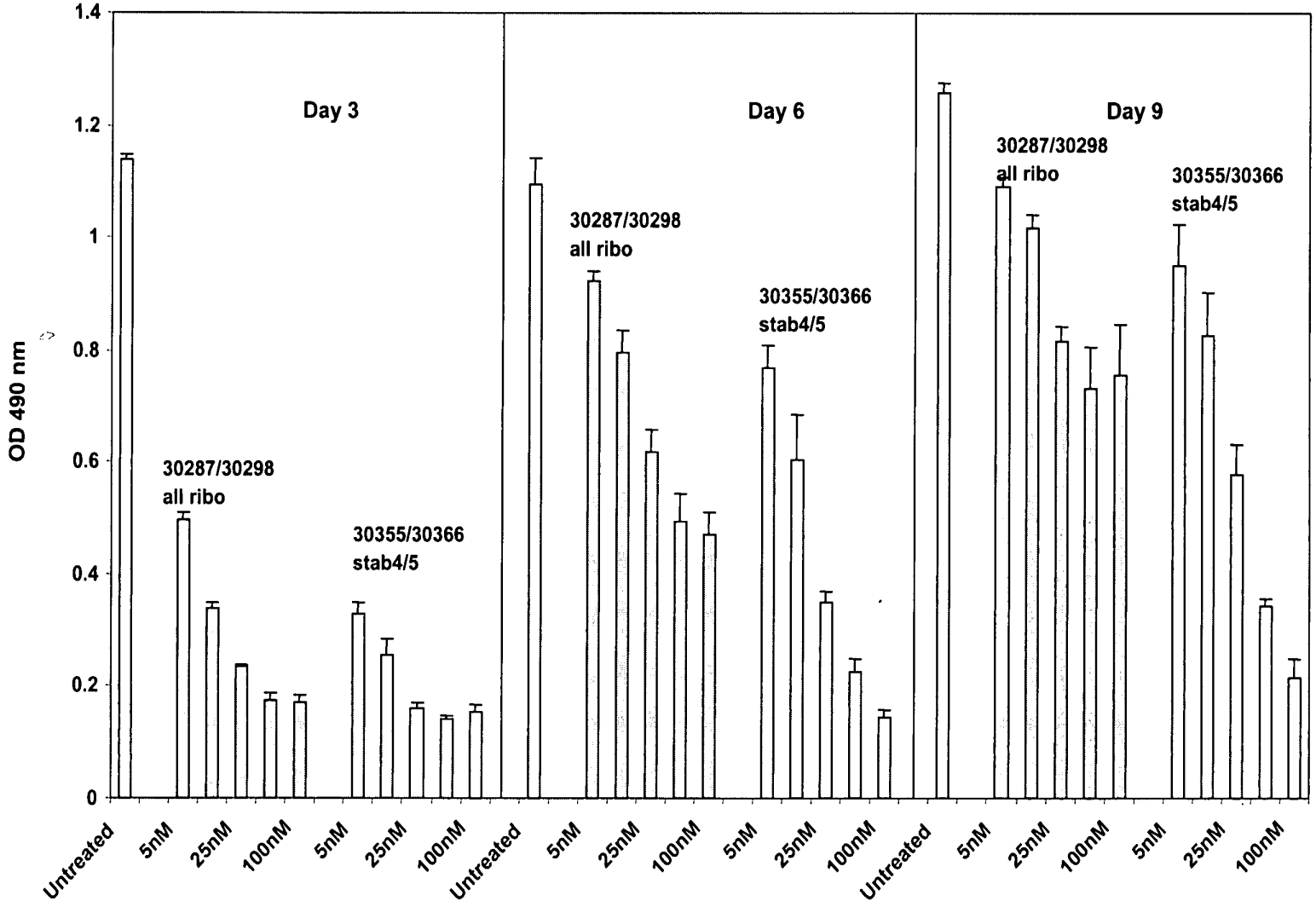


Figure 29: Duration of siRNA Effect
All-Ribo vs. Stab7/8 HBV Site 1580: HBsAg Levels

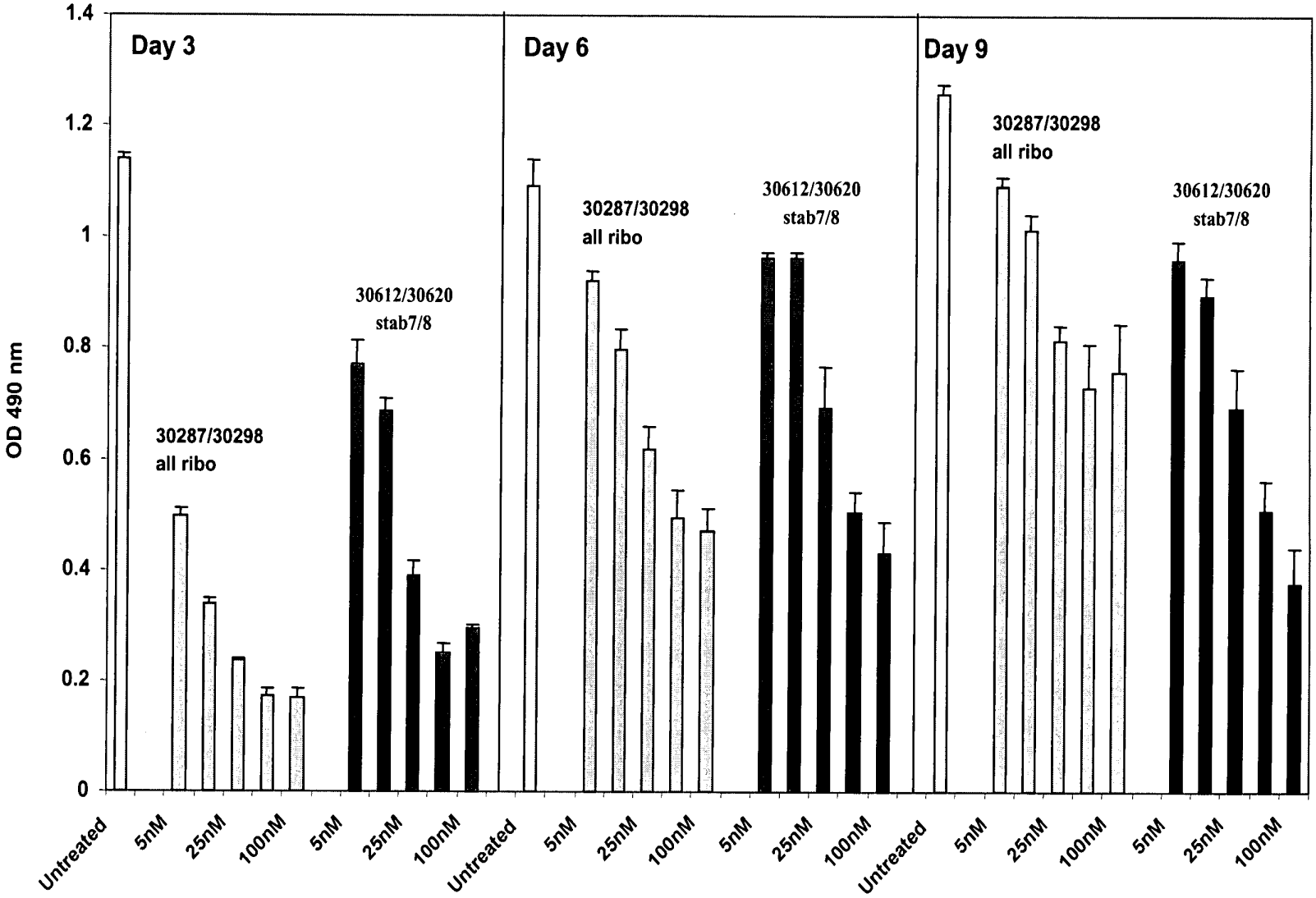


Figure 30: Duration of siRNA Effect
All-Ribo vs. Stab7/11 HBV Site 1580: HBsAg Levels

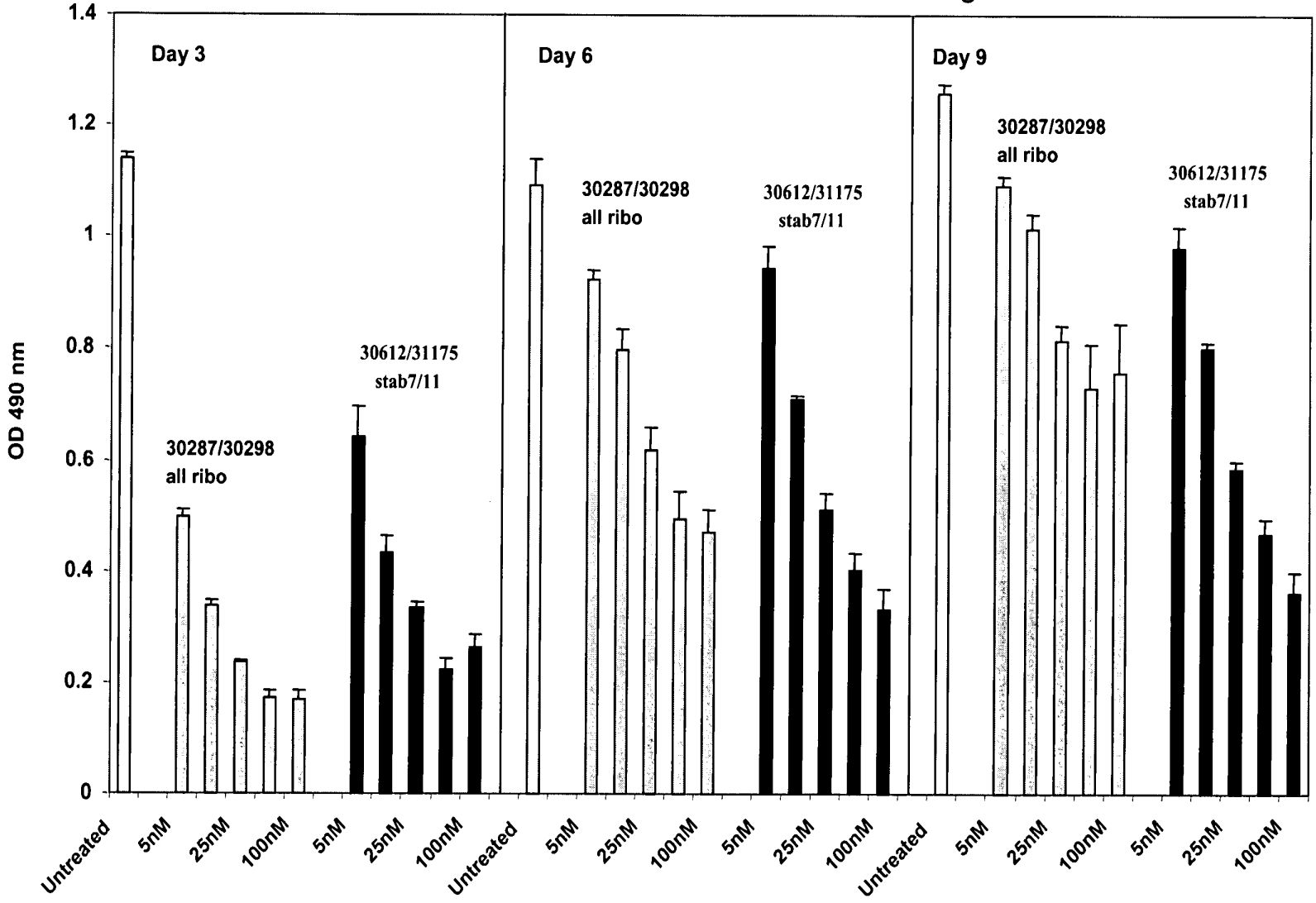


Figure 31: Duration of siRNA Effect
All-Ribo vs. Stab9/10 HBV Site 1580: HBsAg Levels

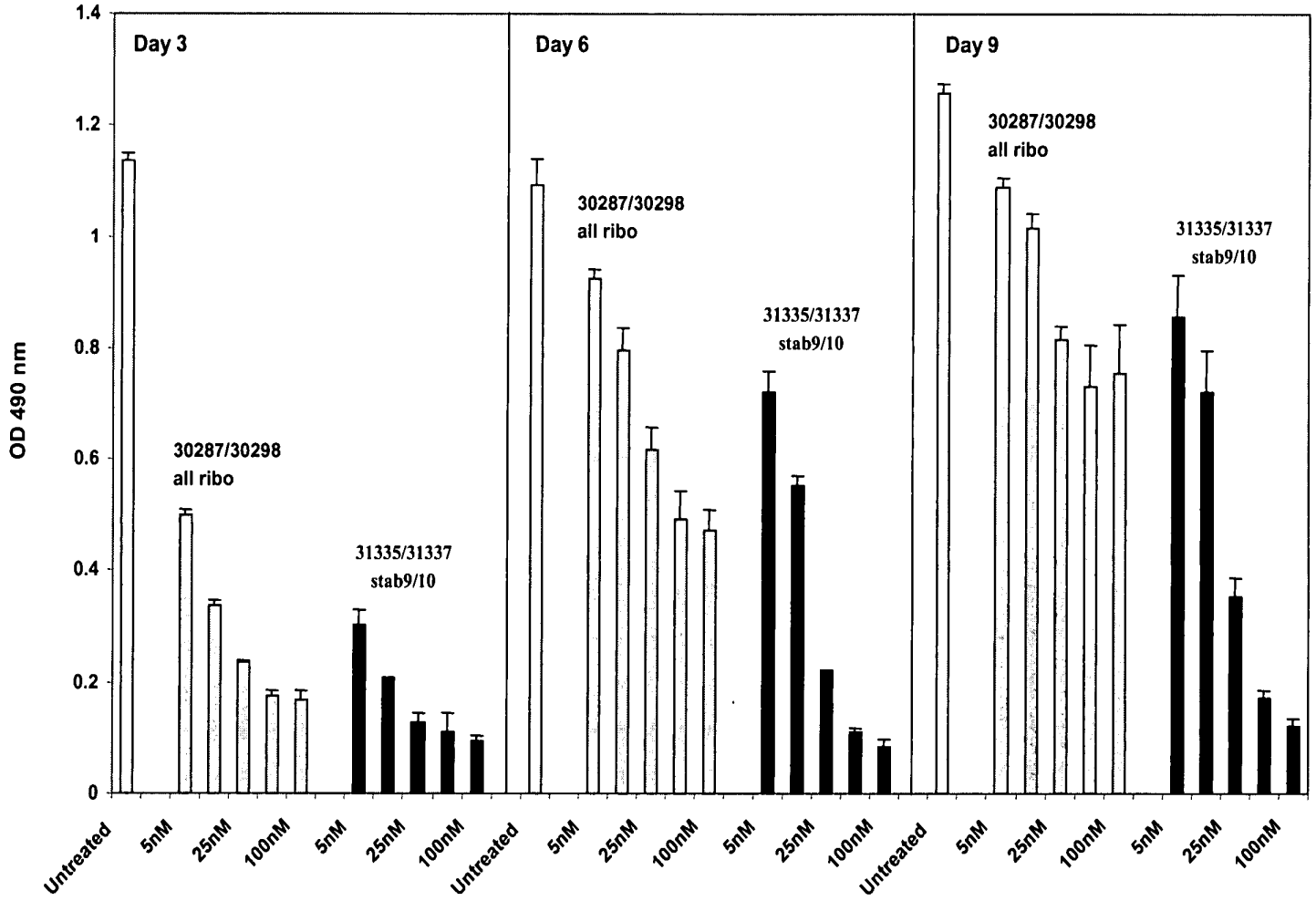


Figure 32 : siRNAs targeting HCV chimera

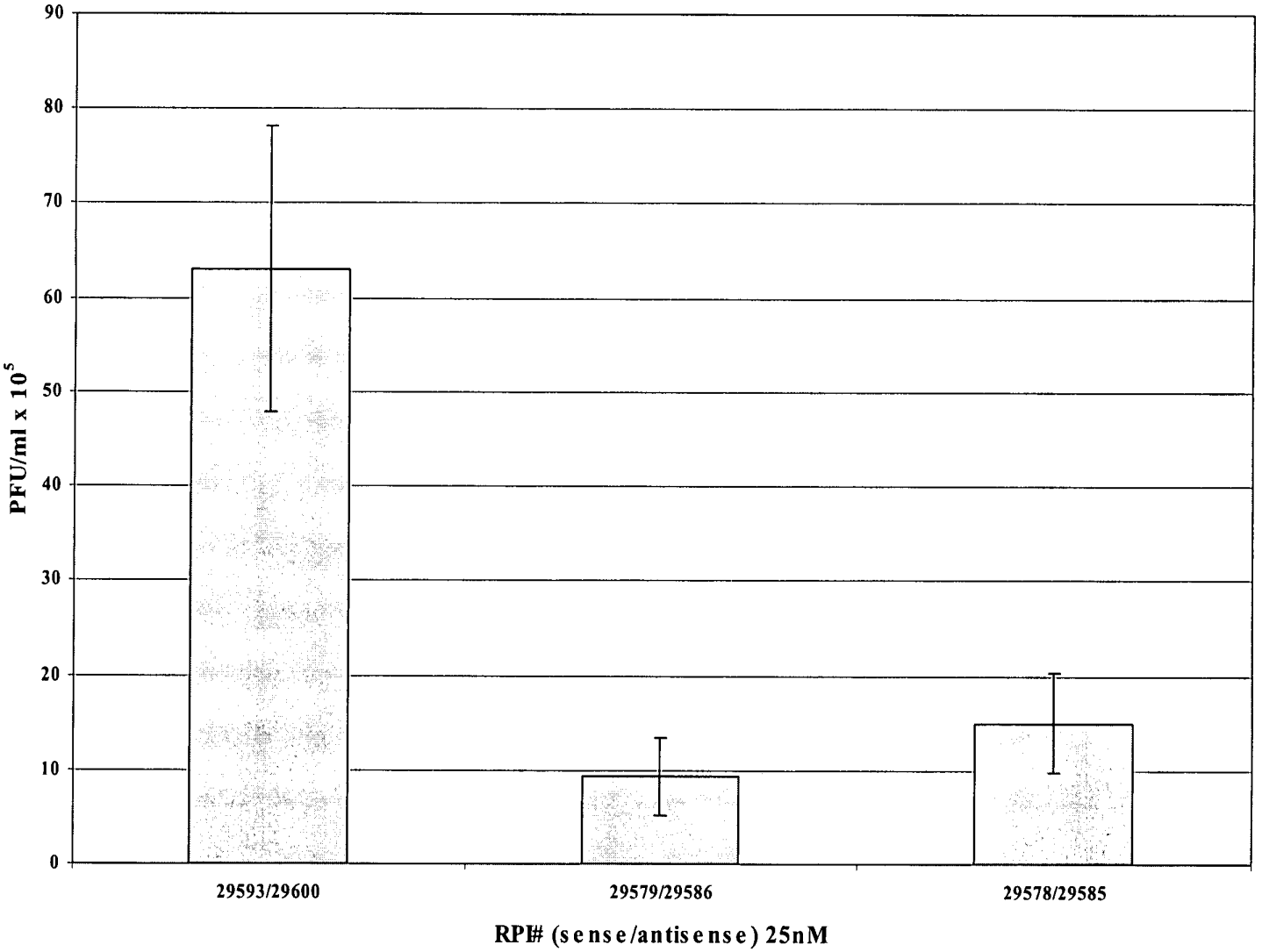


Figure 33: HCV siRNA dose response

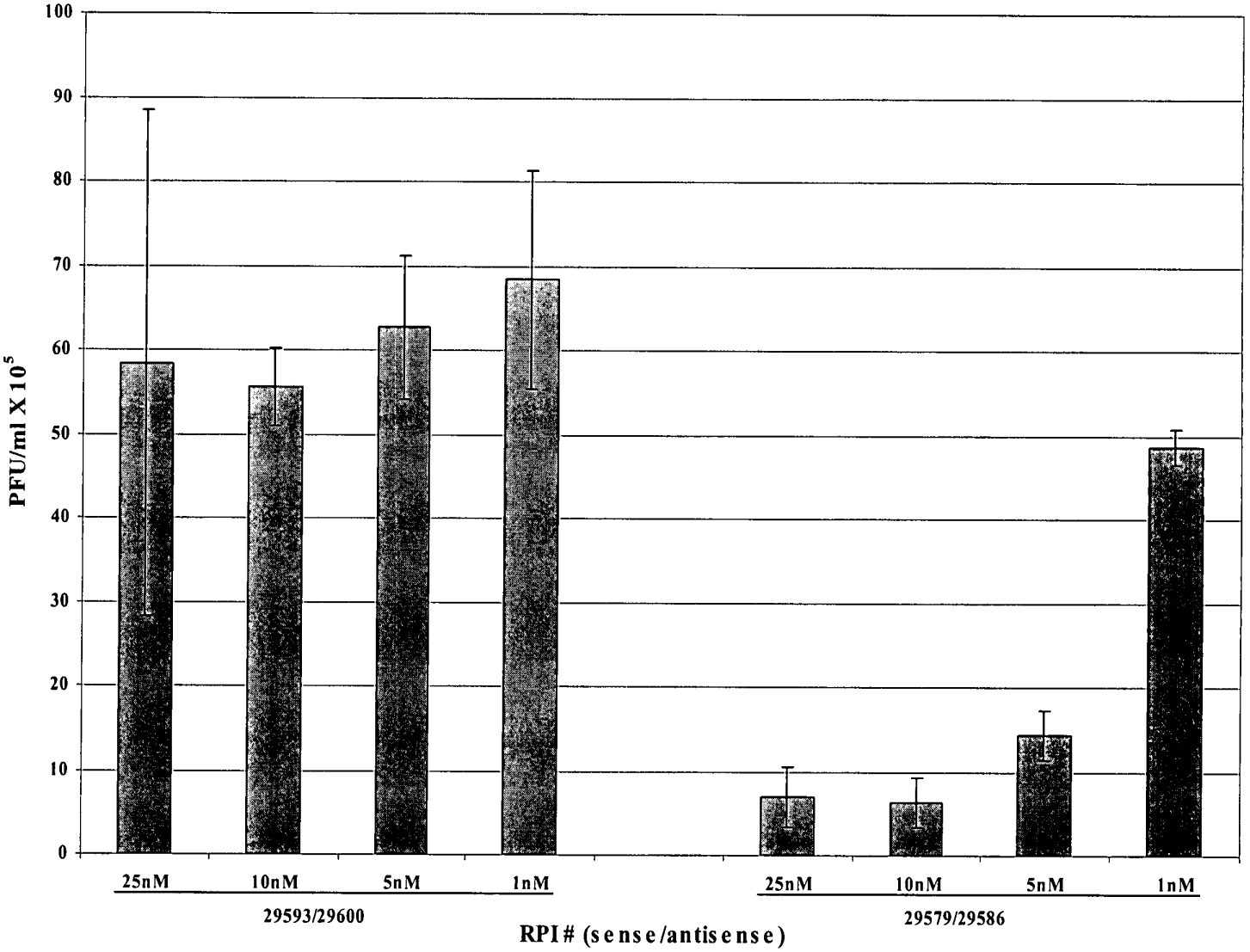


Figure 34: Chemically Modified siRNA targeting HCV chimera

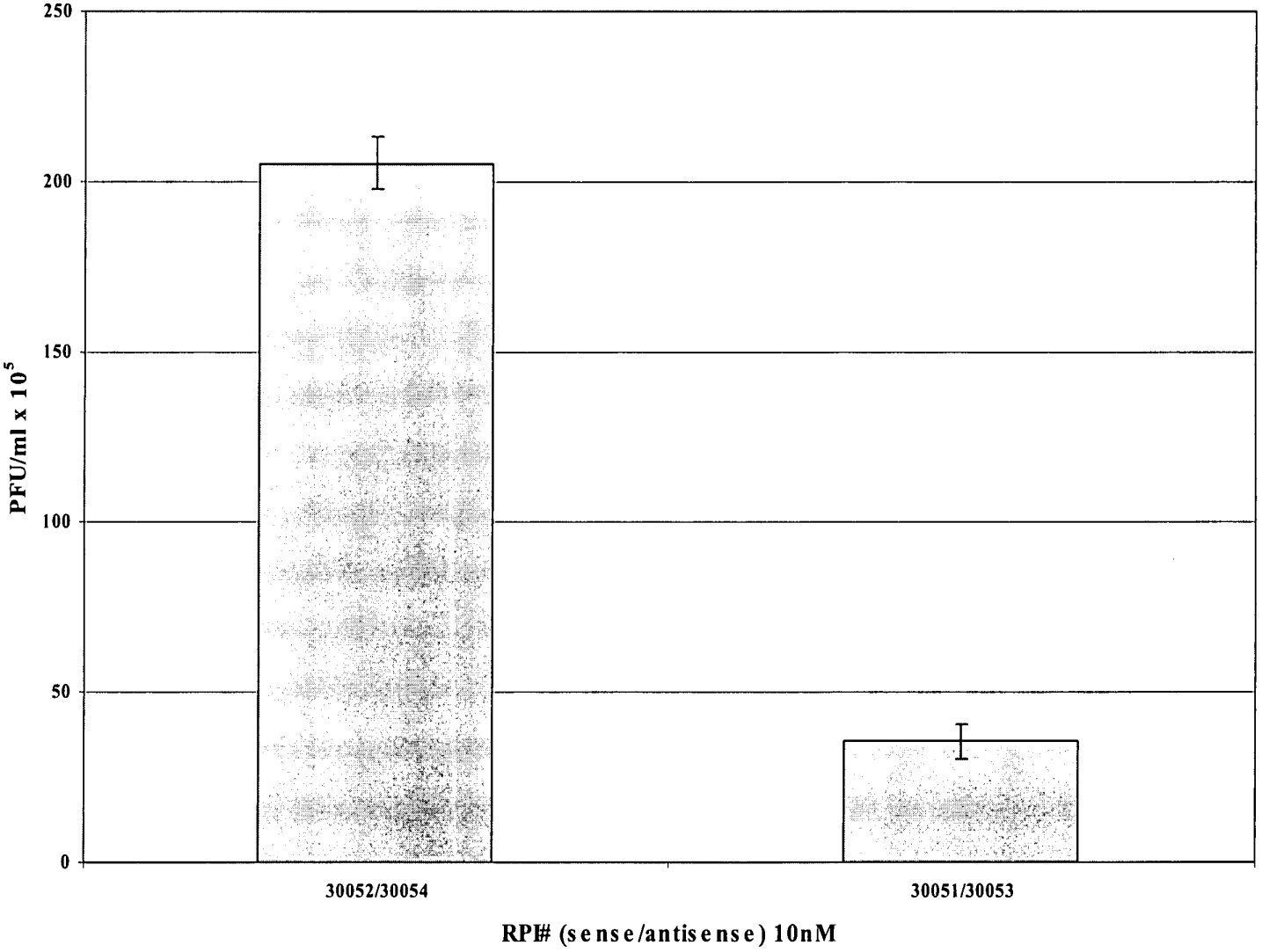


Figure 35: Chemically Modified siRNA targeting HCV chimera

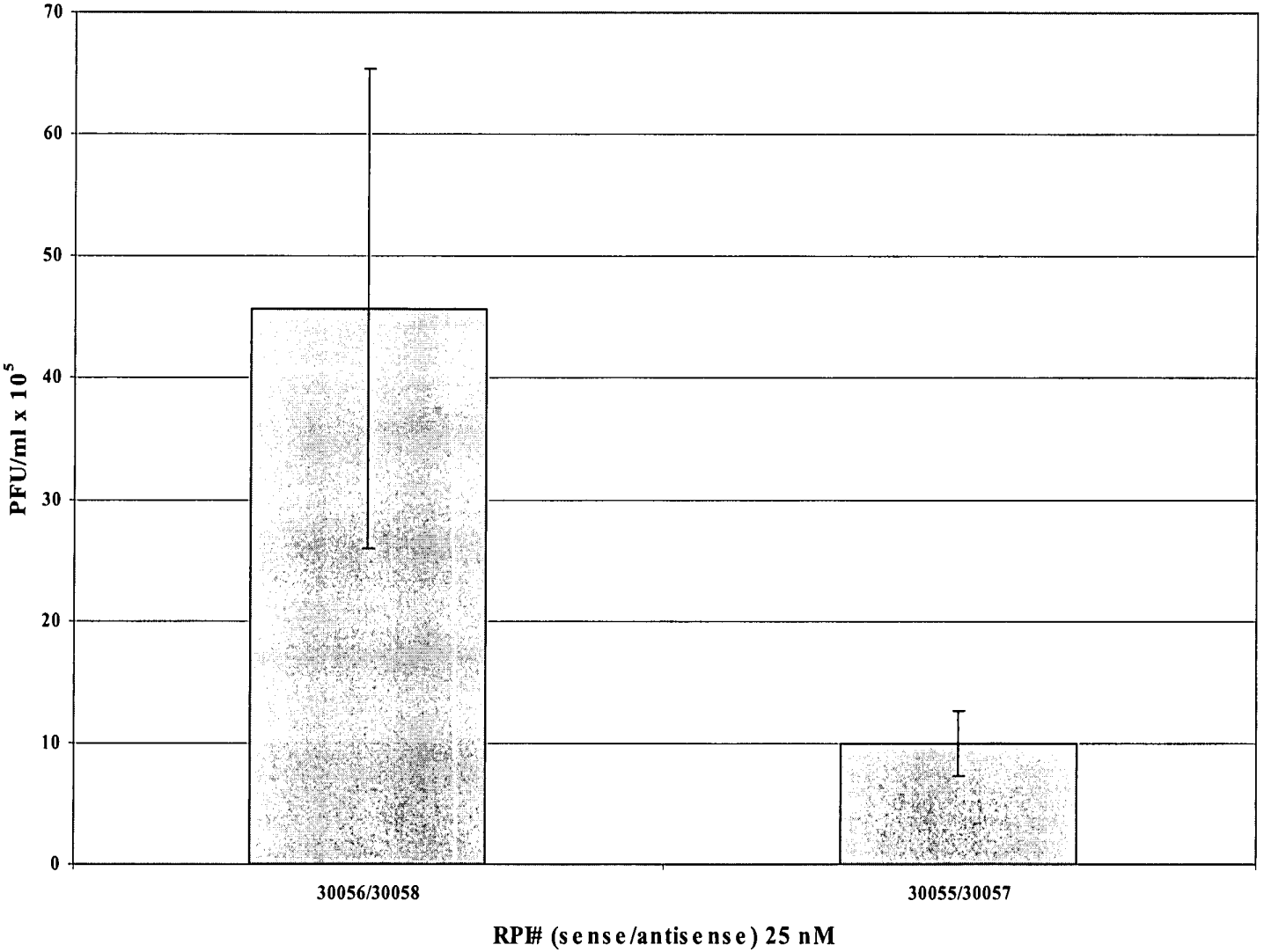


Figure 36: Chemically Modified siRNA targeting HCV chimera

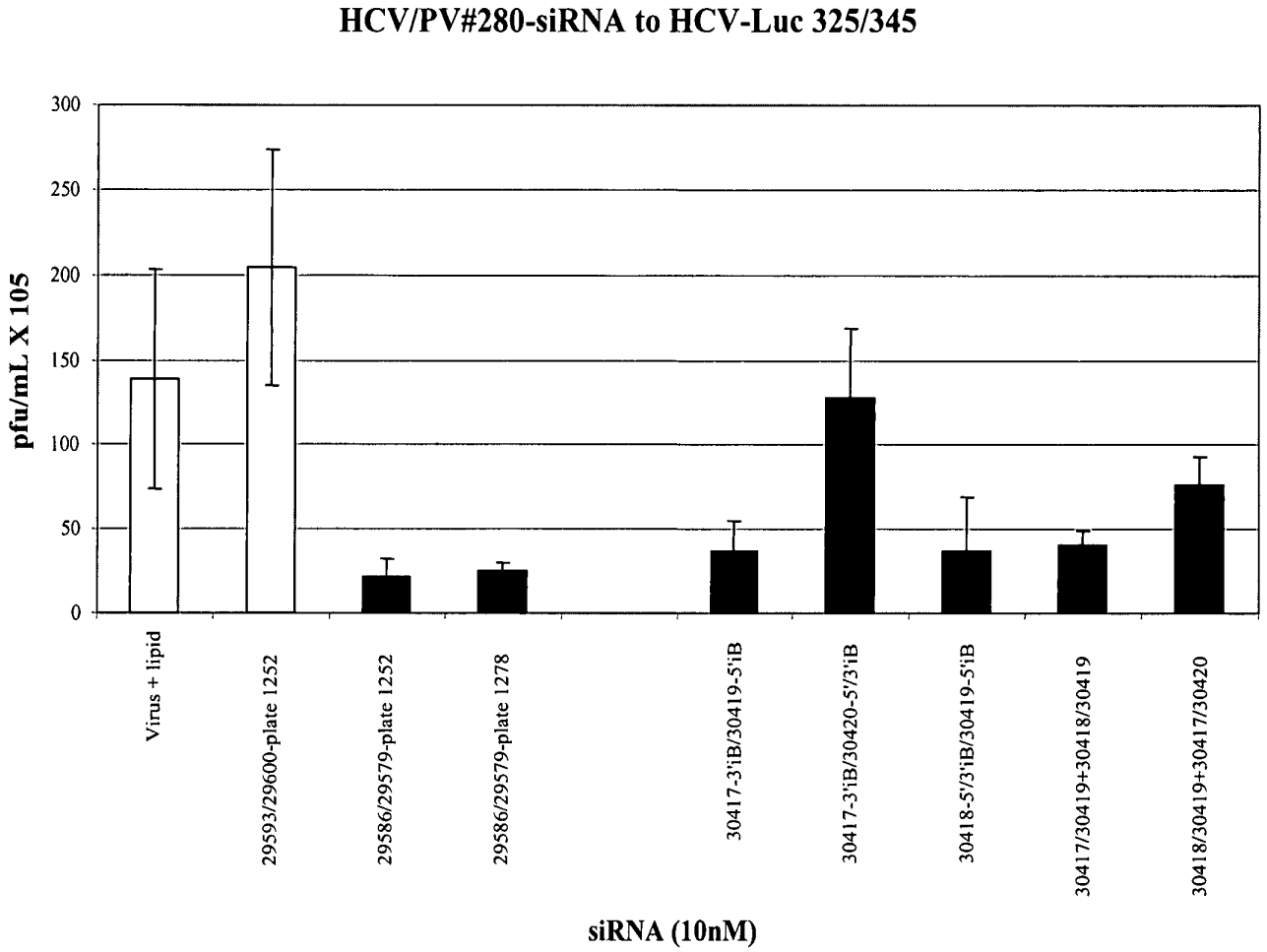


Figure 37: Chemically Modified siRNA targeting HCV chimera

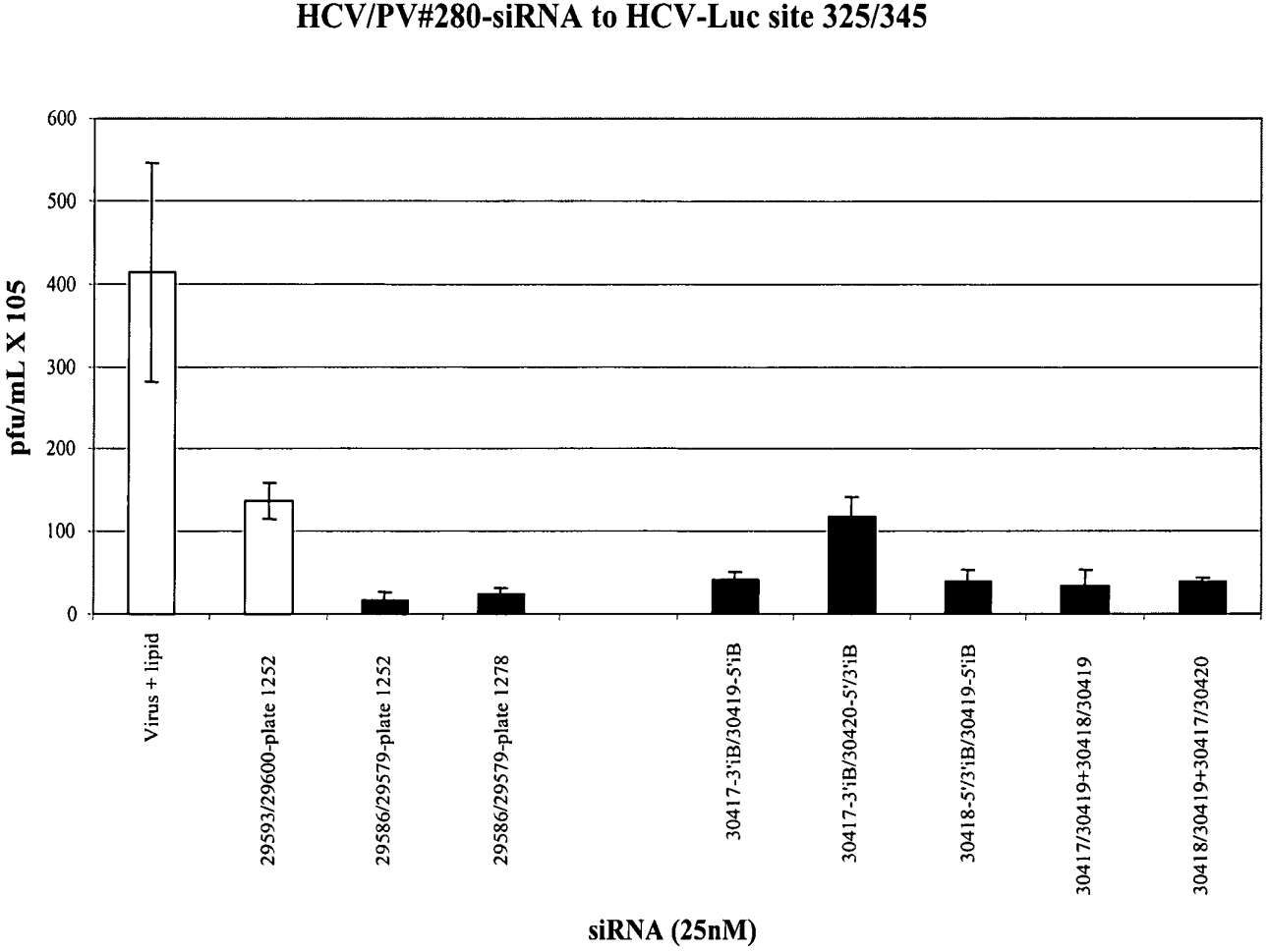


Figure 38: HCV/Replicon Cells transfected with 0.5µl/well LFA 2K-72 hours

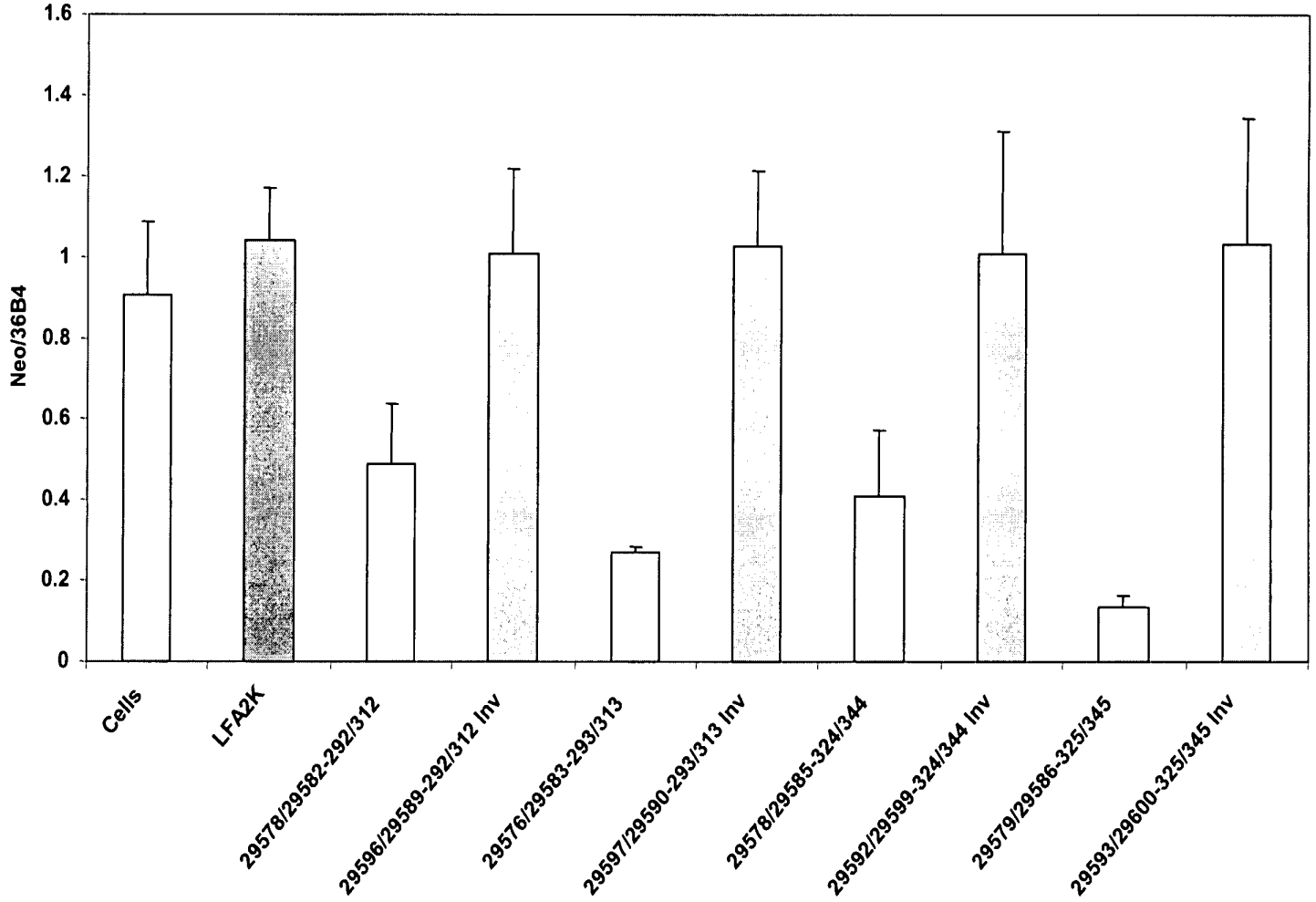


Figure 39: Dose Response with Stab4/5 siNA Leads in HCV Subgenomic Replicon

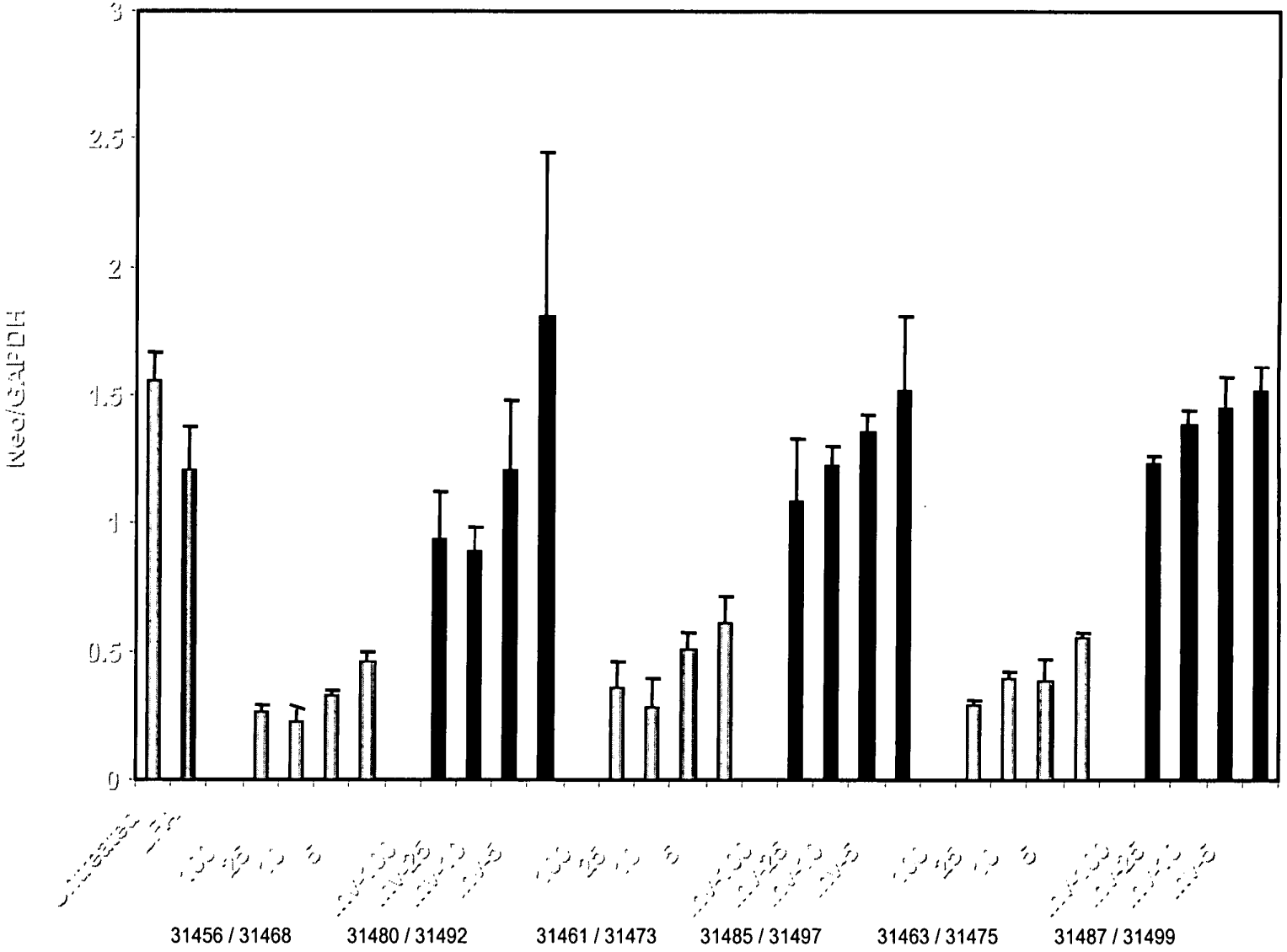


Figure 40: Activity of Stab 7/8 siNA Leads in HCV Subgenomic Replicon

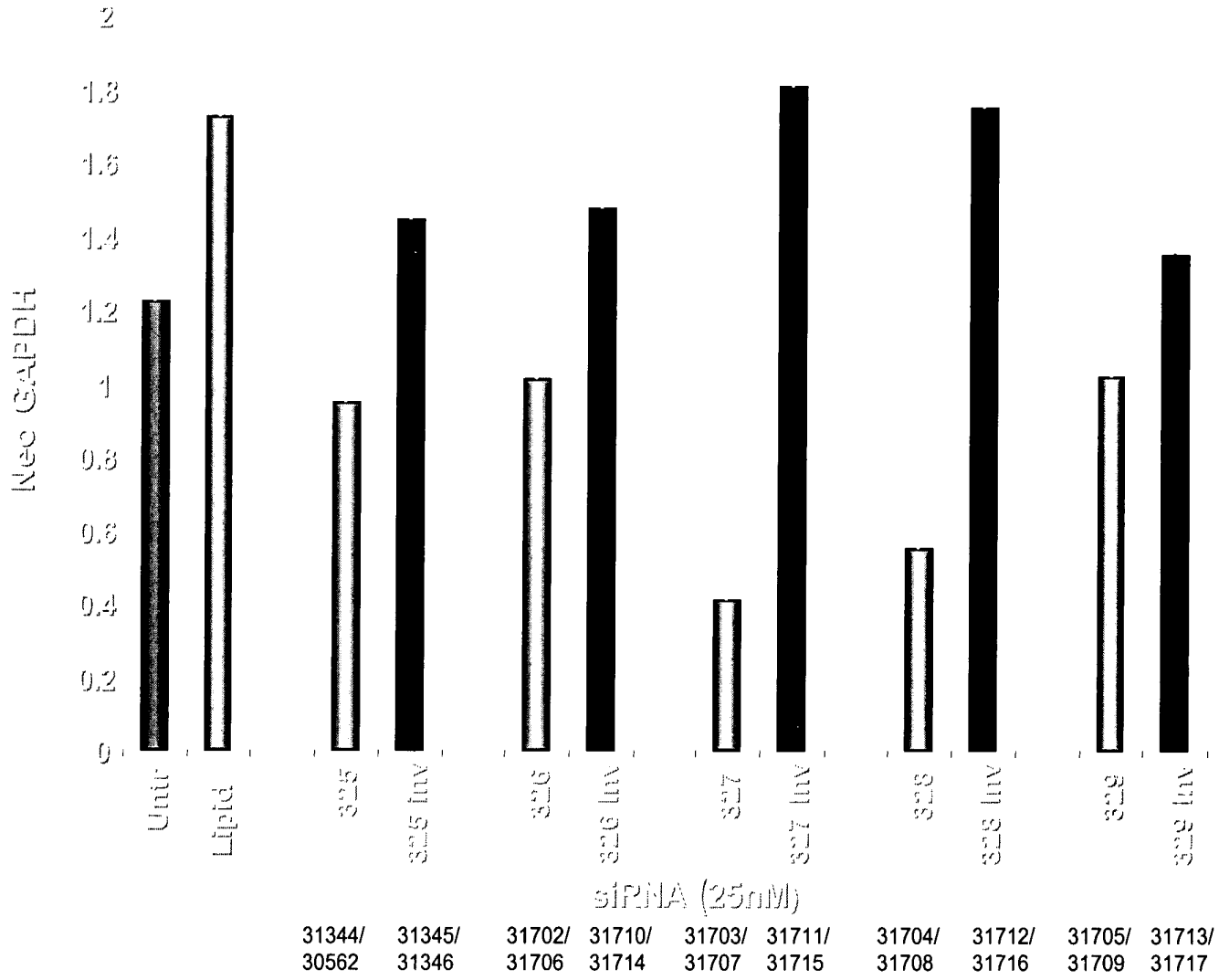


Figure 41: Dose Response with Fully Modified HCV Site 327 siNA

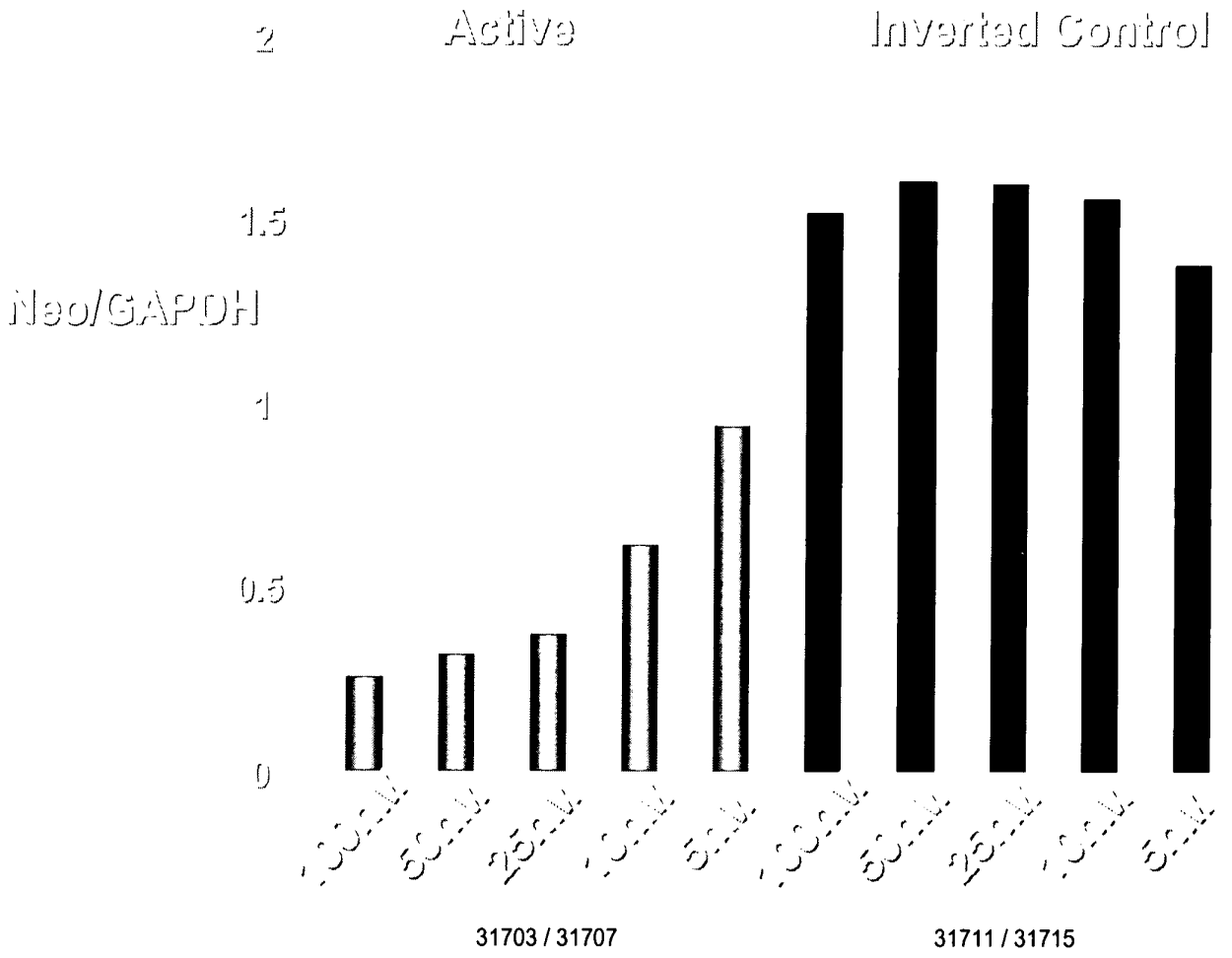


Figure 42: Solid Phase Post-synthetic conjugation of pteroic acid

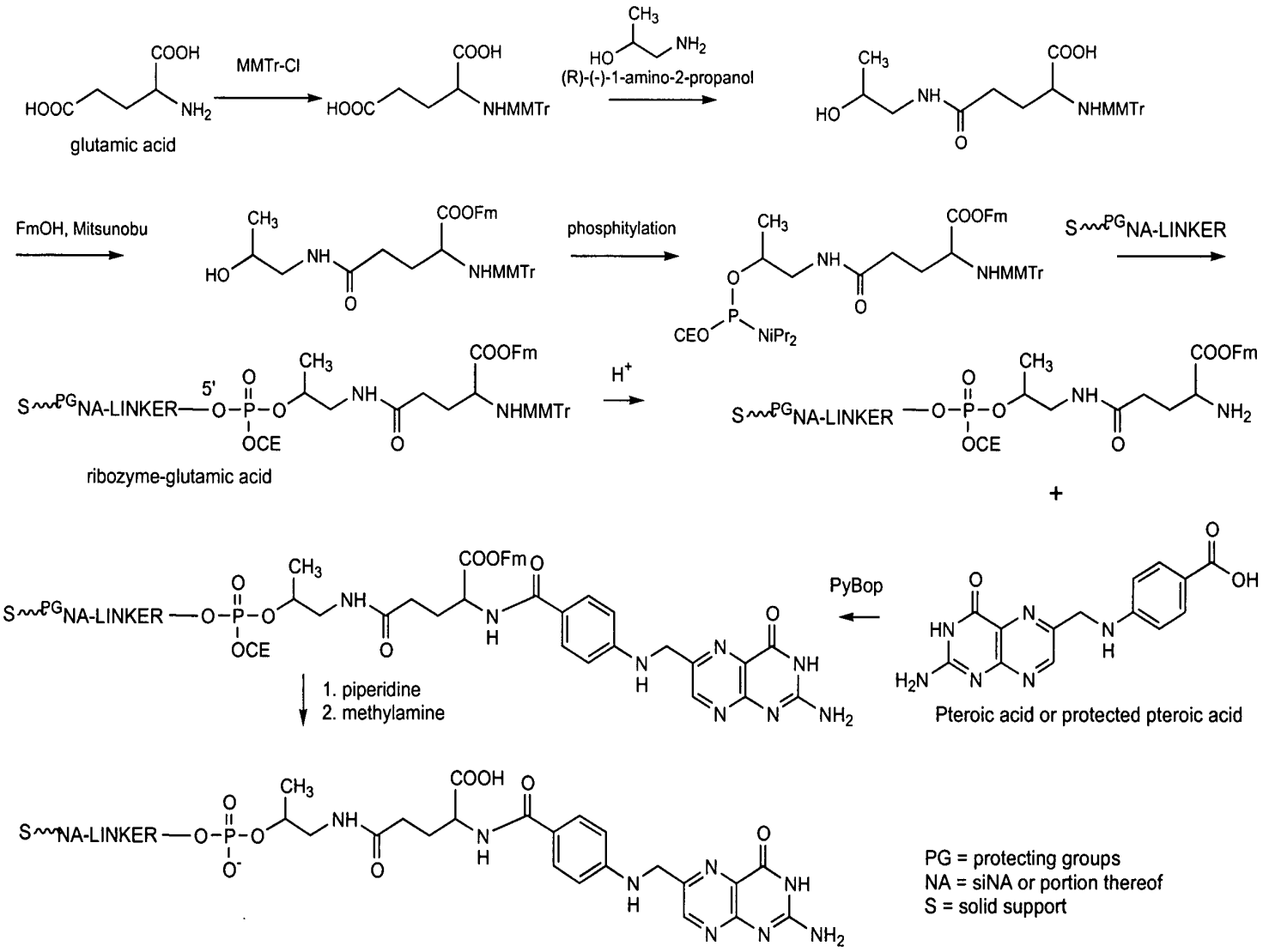
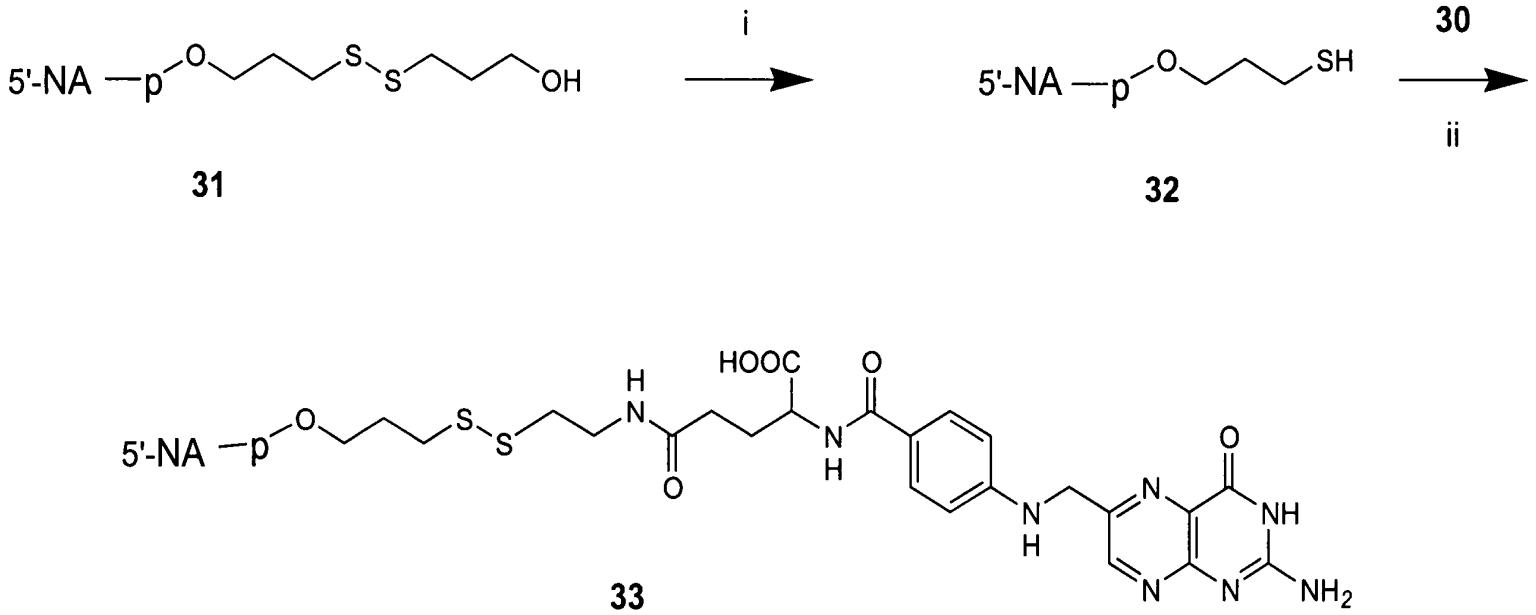
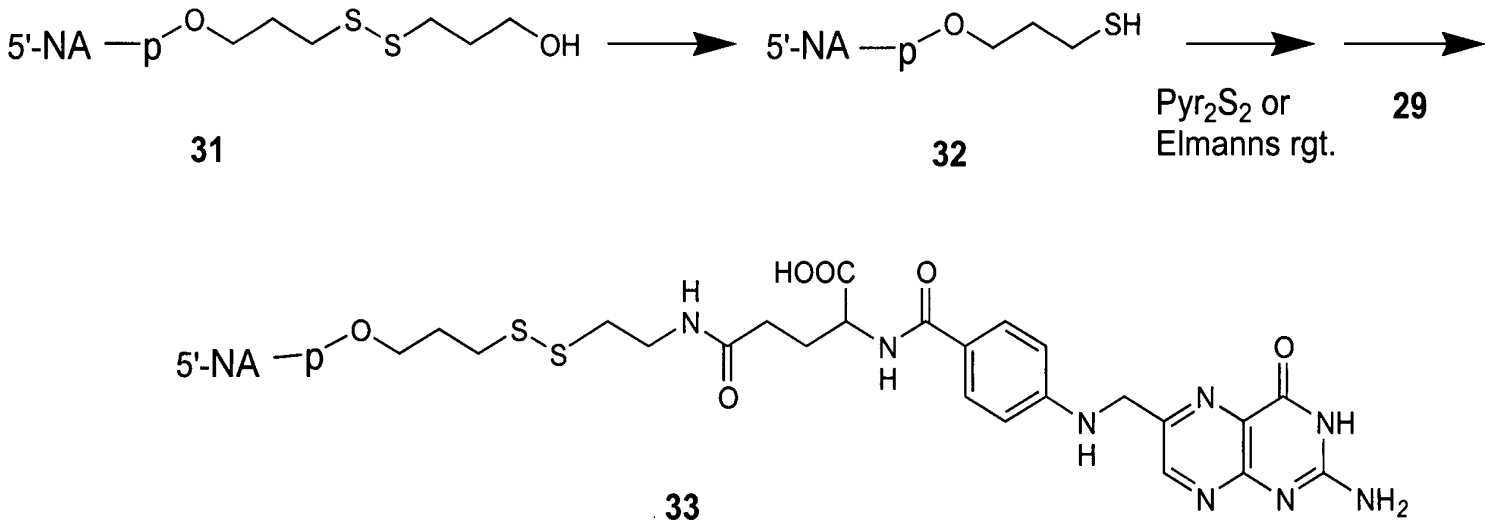


Figure 43



NA = siNA or a portion thereof
p = phosphorous moiety

Figure 44



NA = siNA or a portion thereof
p = phosphorous moiety

Figure 45: Solid Phase Post-synthetic conjugation of pteroic acid

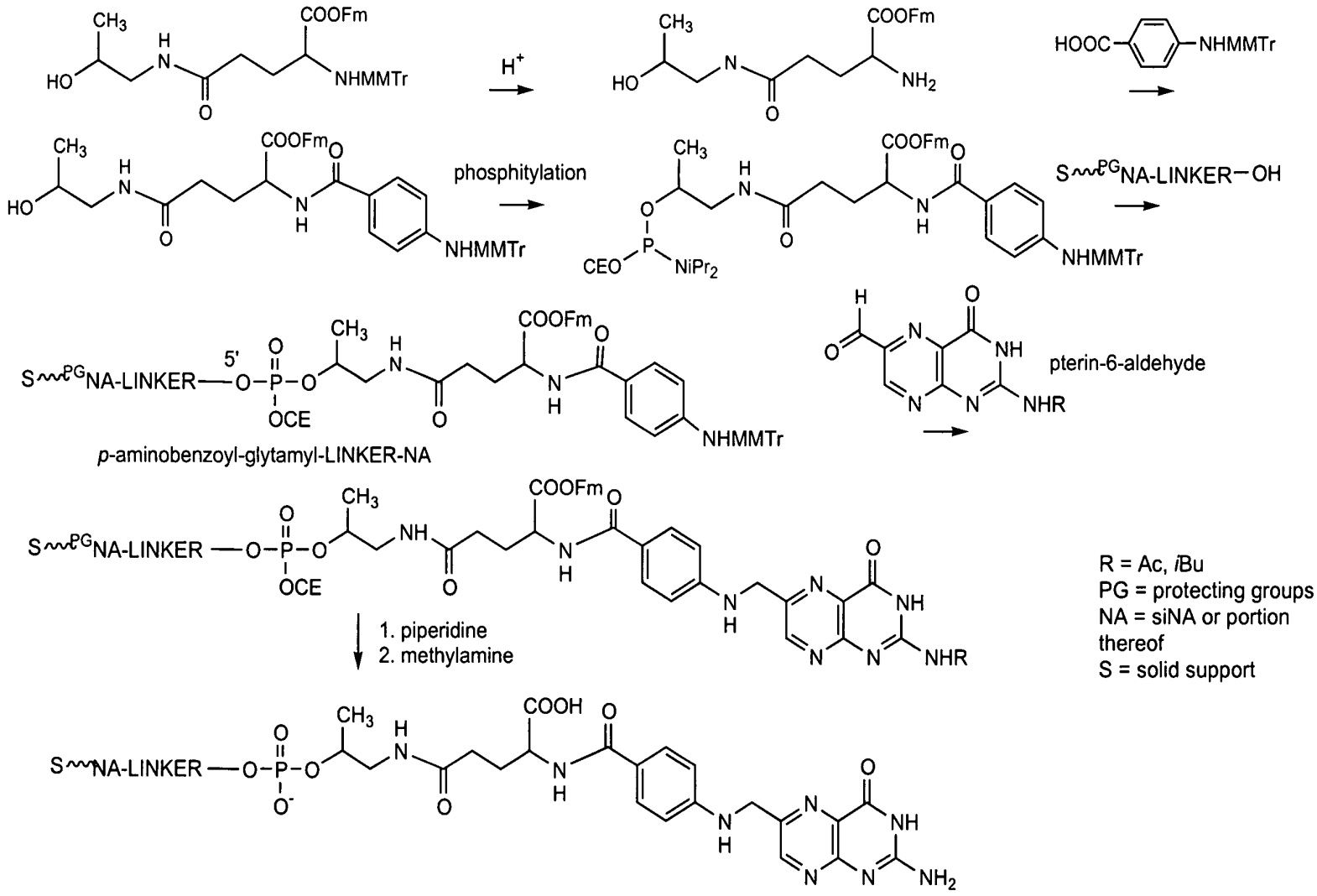
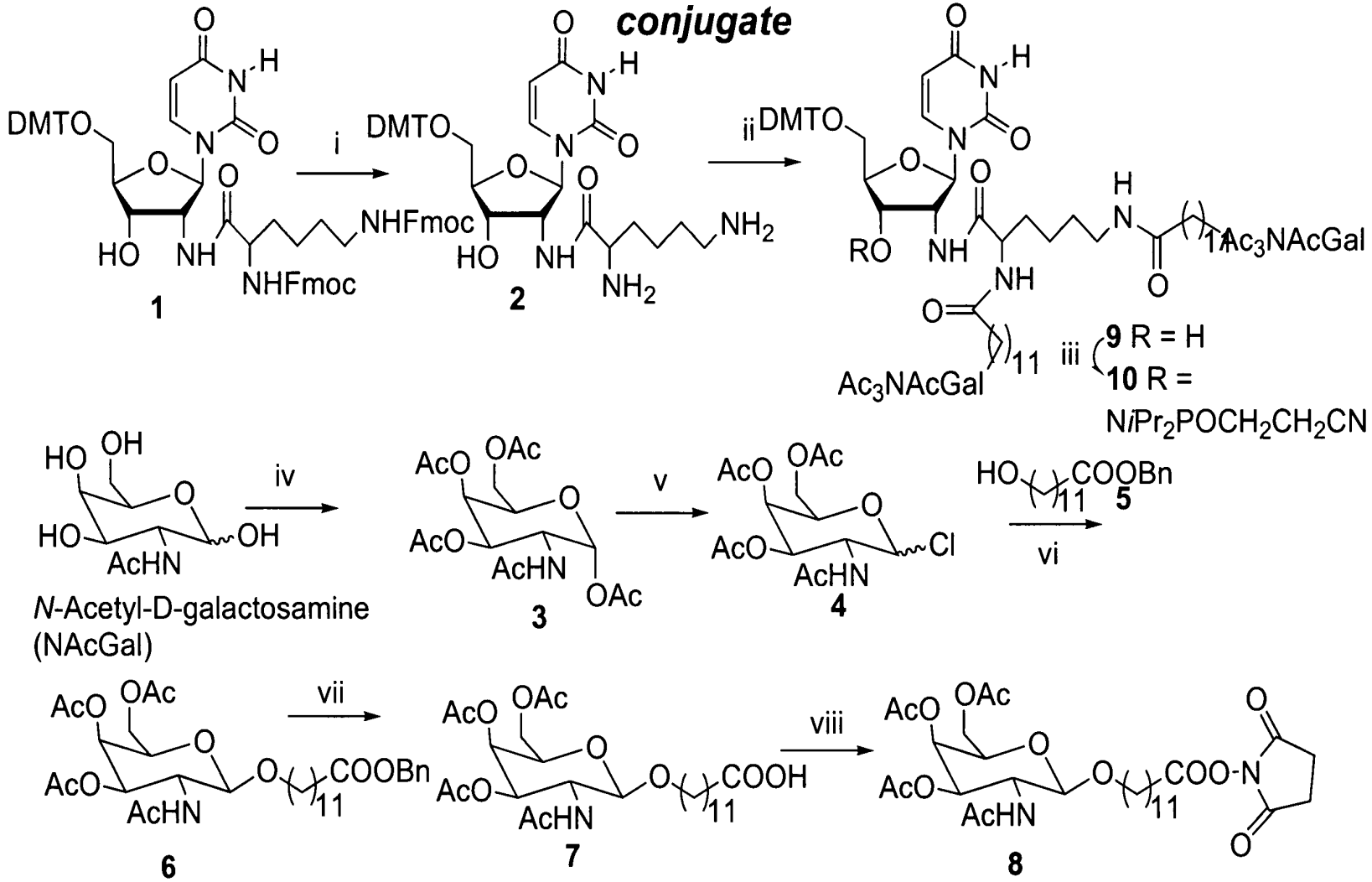
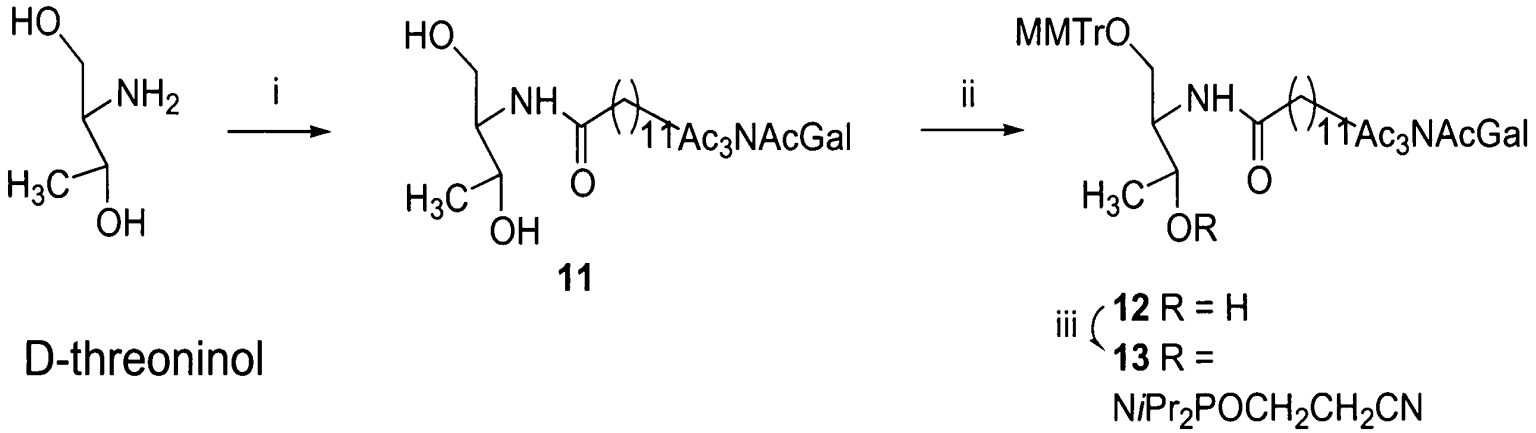


Figure 46: Synthesis of N-acetyl-D-galactosamine-2'-aminouridine conjugate



Reagents and Conditions: (i) diethylamine, DMF, (ii) **8**, diisopropylethylamine, DMF, (iii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH_2Cl_2, (iv) Ac_2O, TEA, CH_3CN, (v) HCl, Ac_2O, (vi) $Hg(CN)_2$, MS 4A, CH_3NO_2-toluene 1:1, (vii) H_2, 5% Pd-C, ethanol, (viii) *N*-hydroxysuccinimide, DCC, THF.

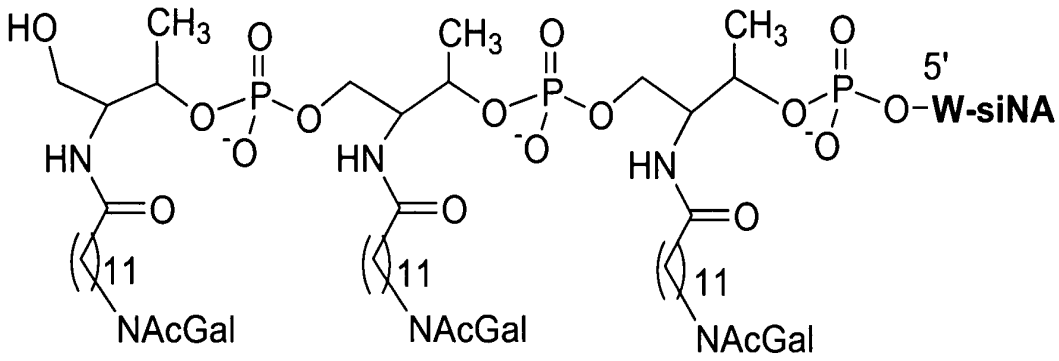
Figure 47: Synthesis of N-acetyl-D-galactosamine-D-threoninol conjugate



D-threoninol

Reagents and Conditions: (i) **7**, DCC, *N*-hydroxysuccinimide, (ii) MMTr-Cl, pyridine, (iii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH_2Cl_2.

Figure 48: Conjugation of targeting ligands to the 5'-end of a siNA molecule



N-acetyl-D-galactosamine conjugate

Figure 49: Synthesis of dodecanoic acid linker

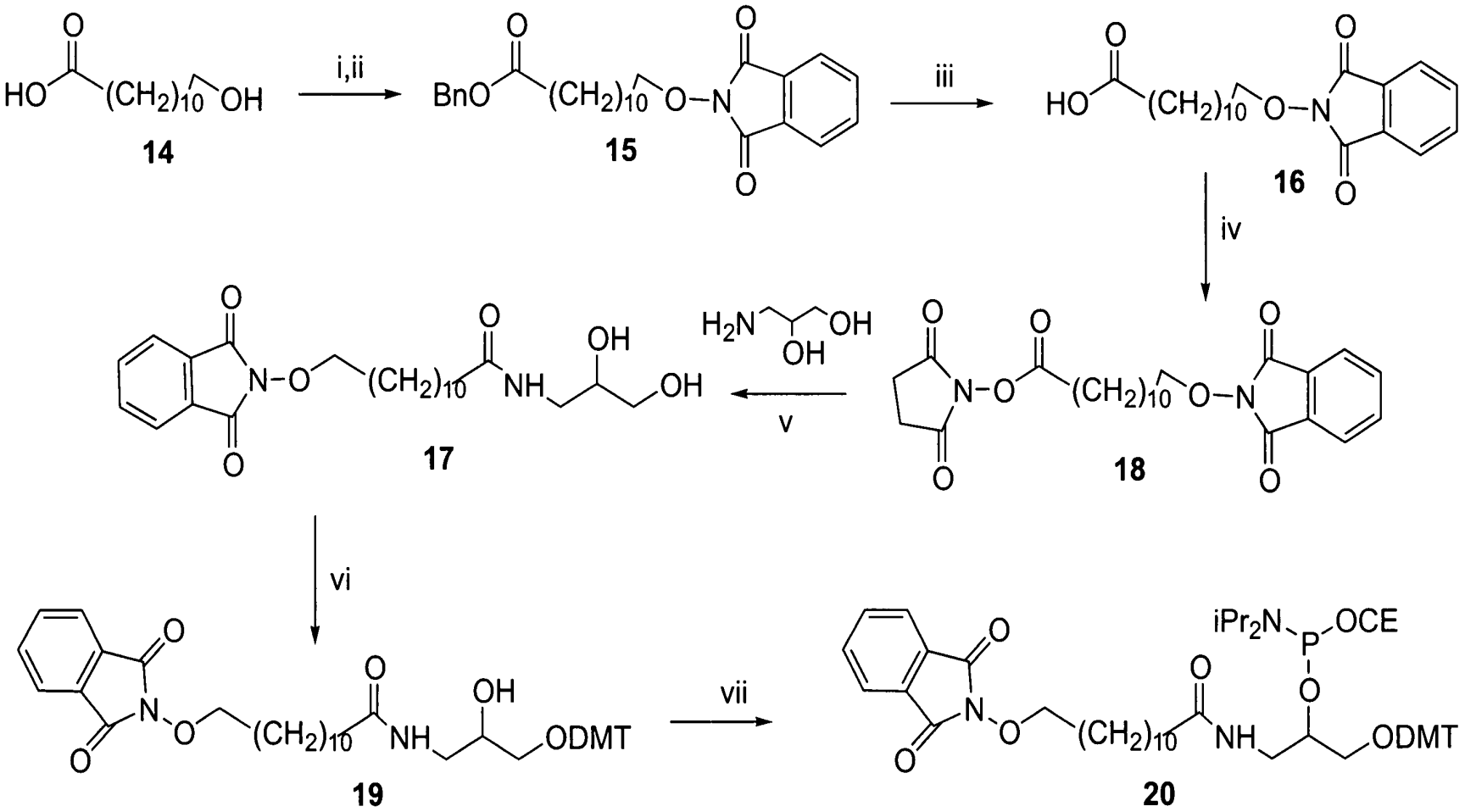


Figure 50: Oxime linked siNA/Peptide Conjugate

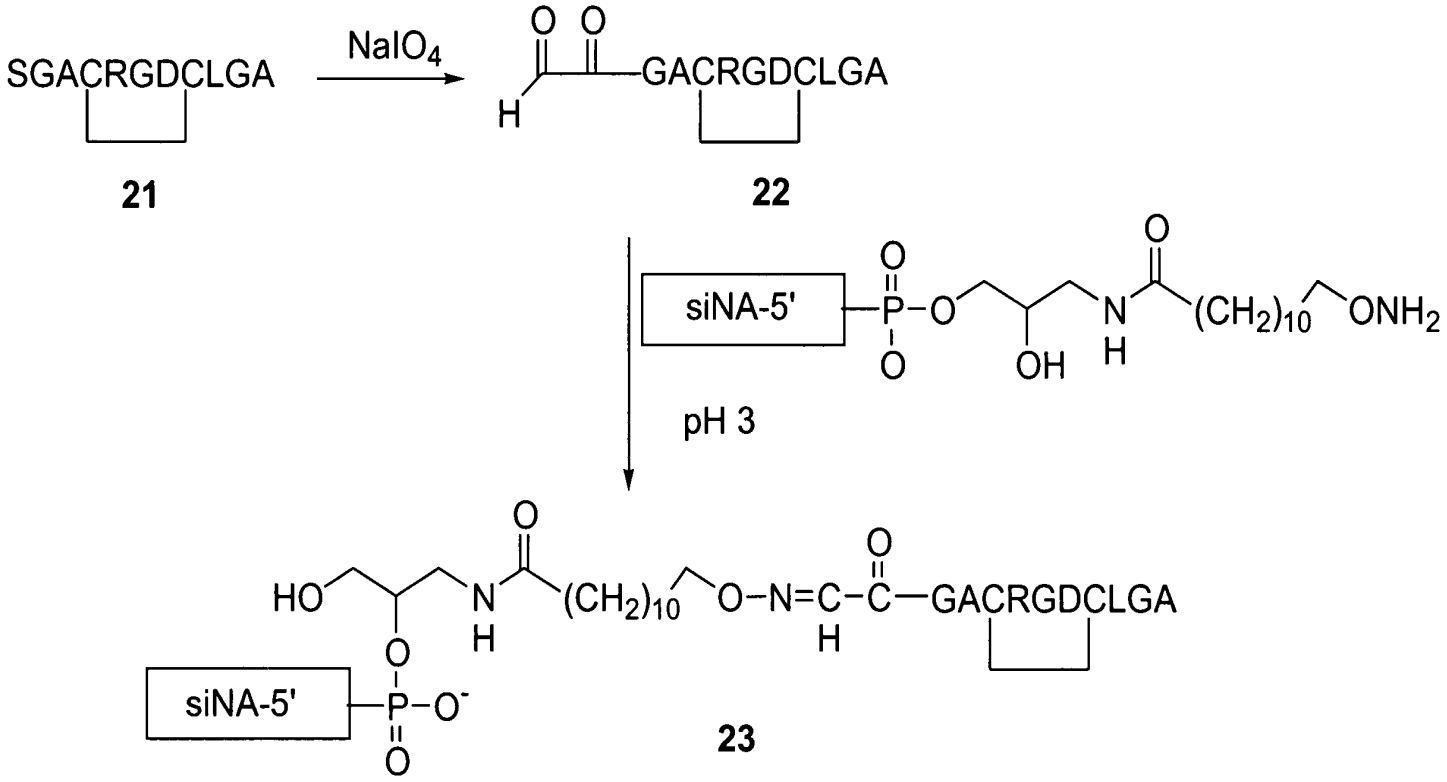
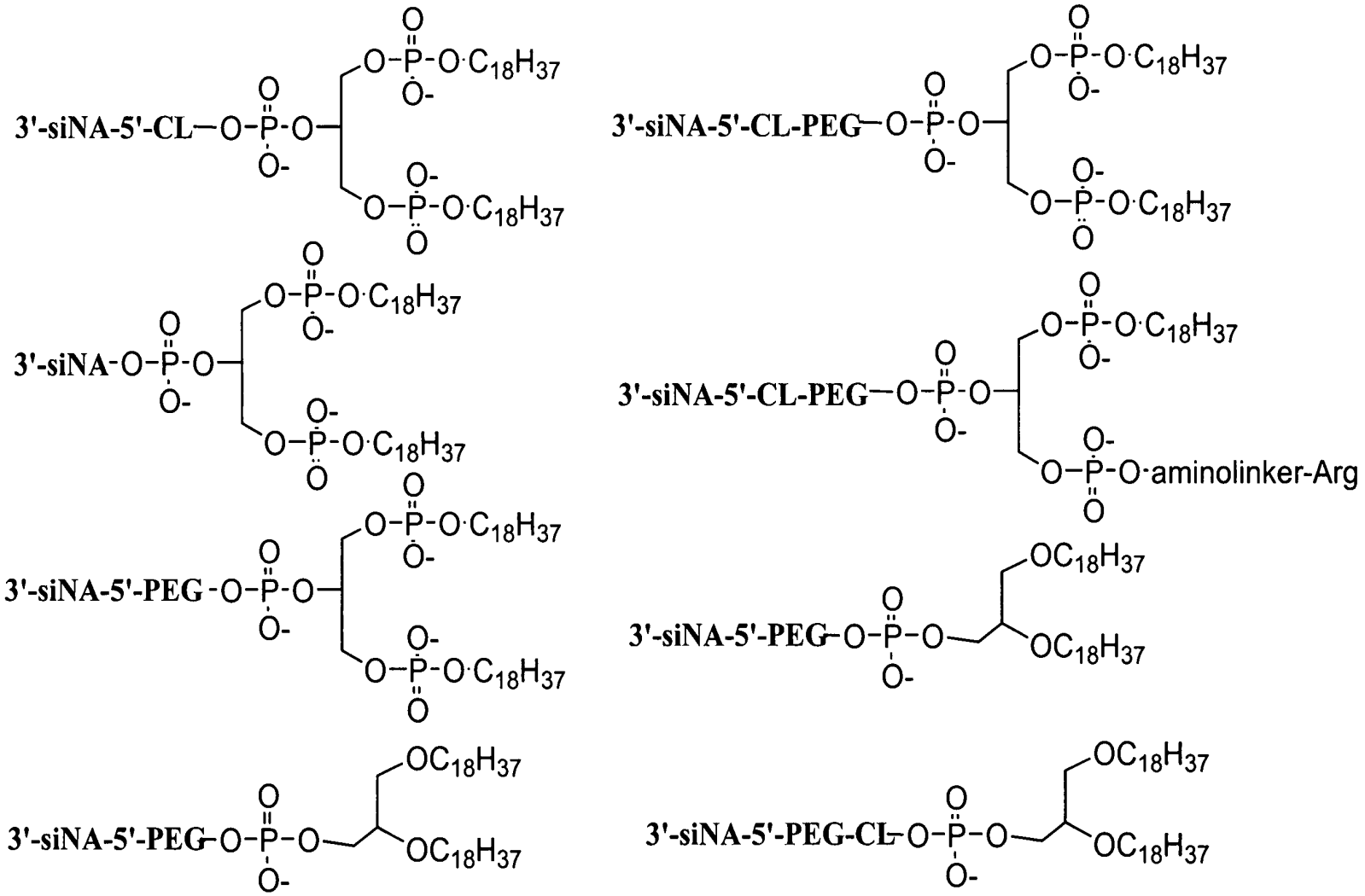
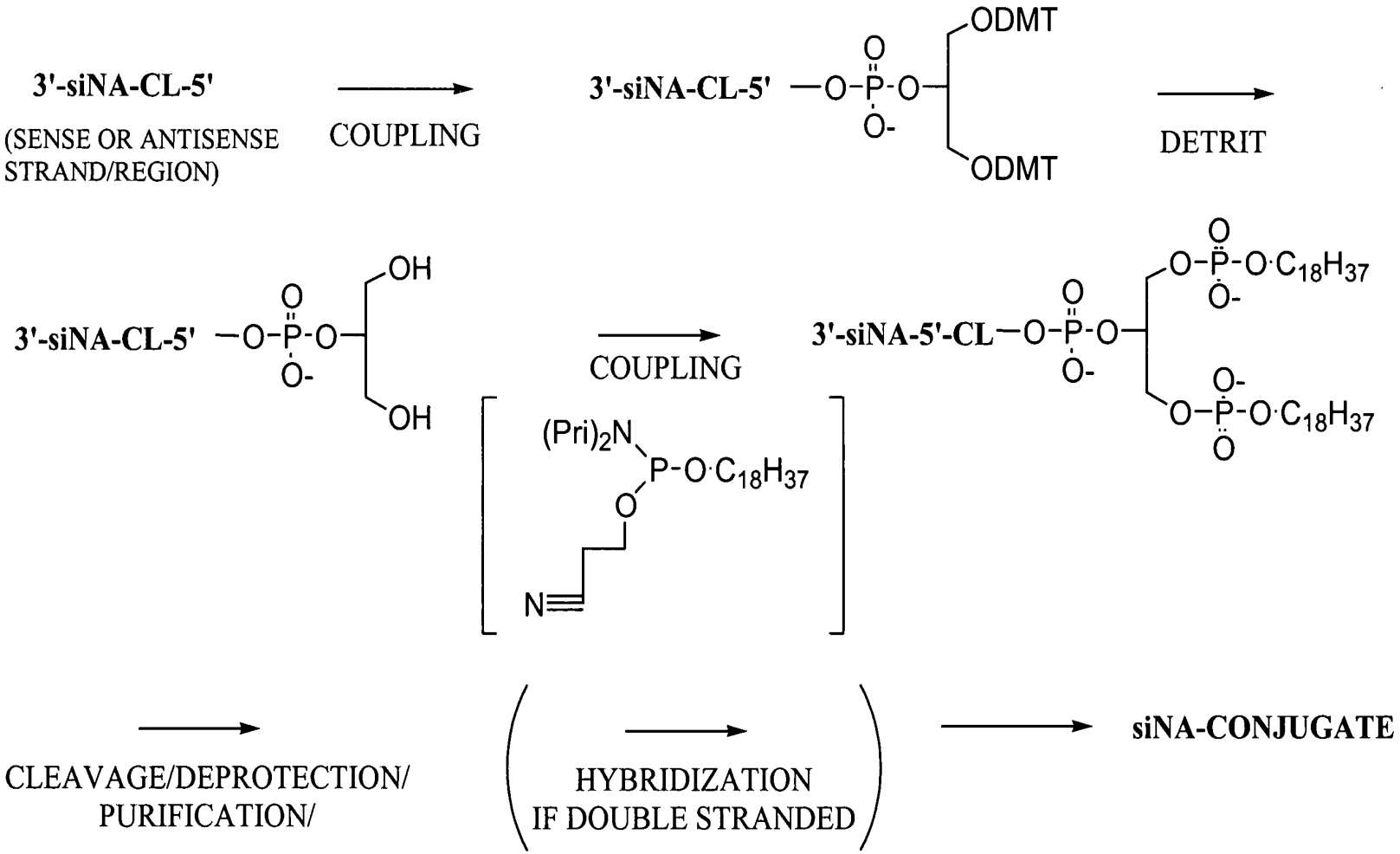


Figure 51: siNA/Phospholipid Conjugates



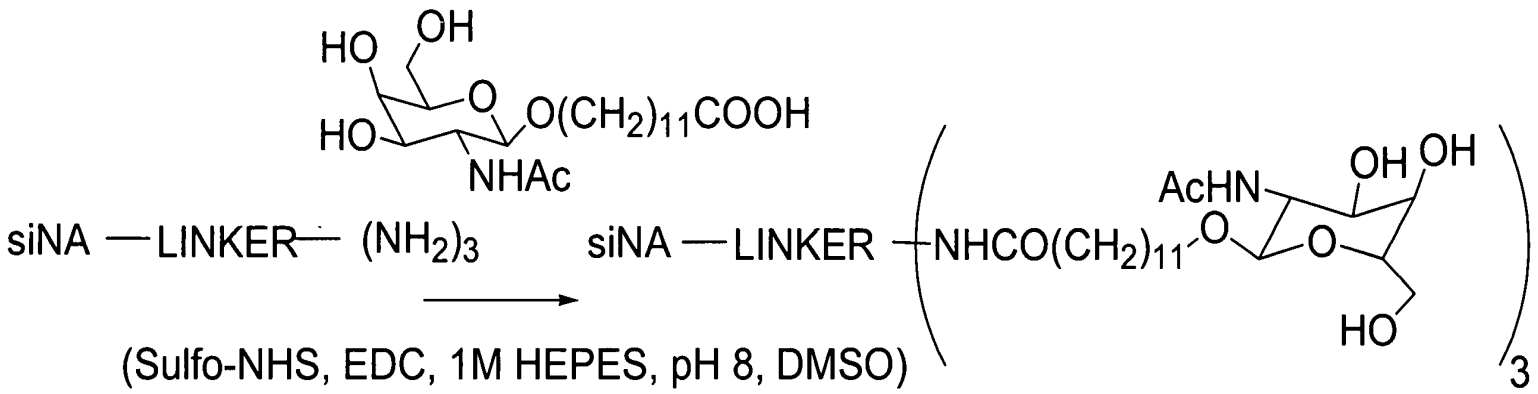
PEG=polyethylene glycol
CL=cleavable linker (e.g. A-dT, C-dT)
siNA= short interfering nucleic acid molecule or a portion thereof

Figure 52: siNA Phospholipid Conjugate

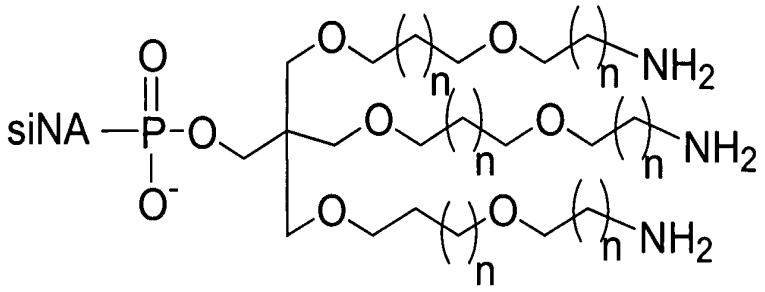


CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT

Figure 53: siNA-NAcGalactosamine post-synthetic coupling

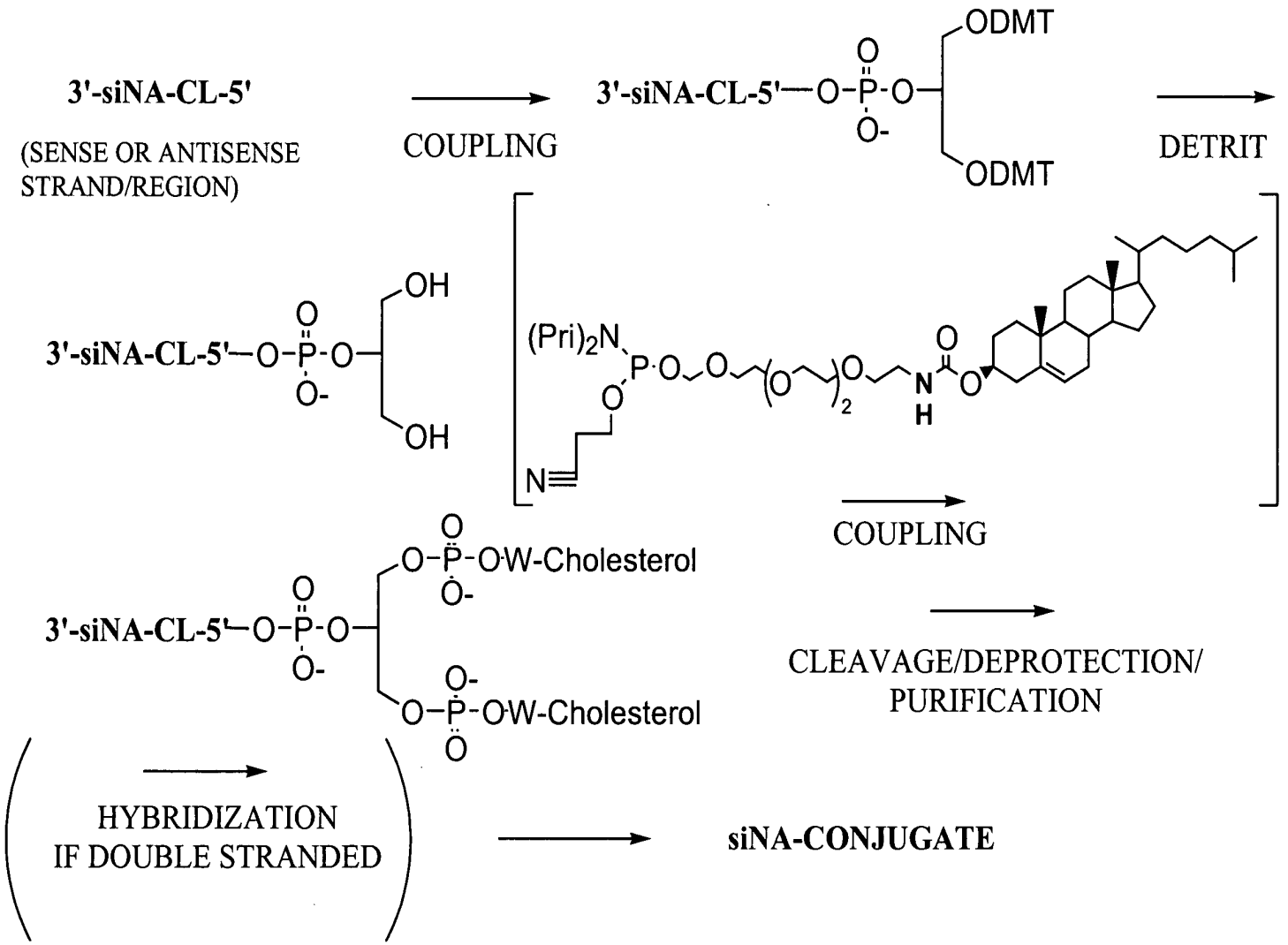


FOR EXAMPLE: OLIGO-LINKER =



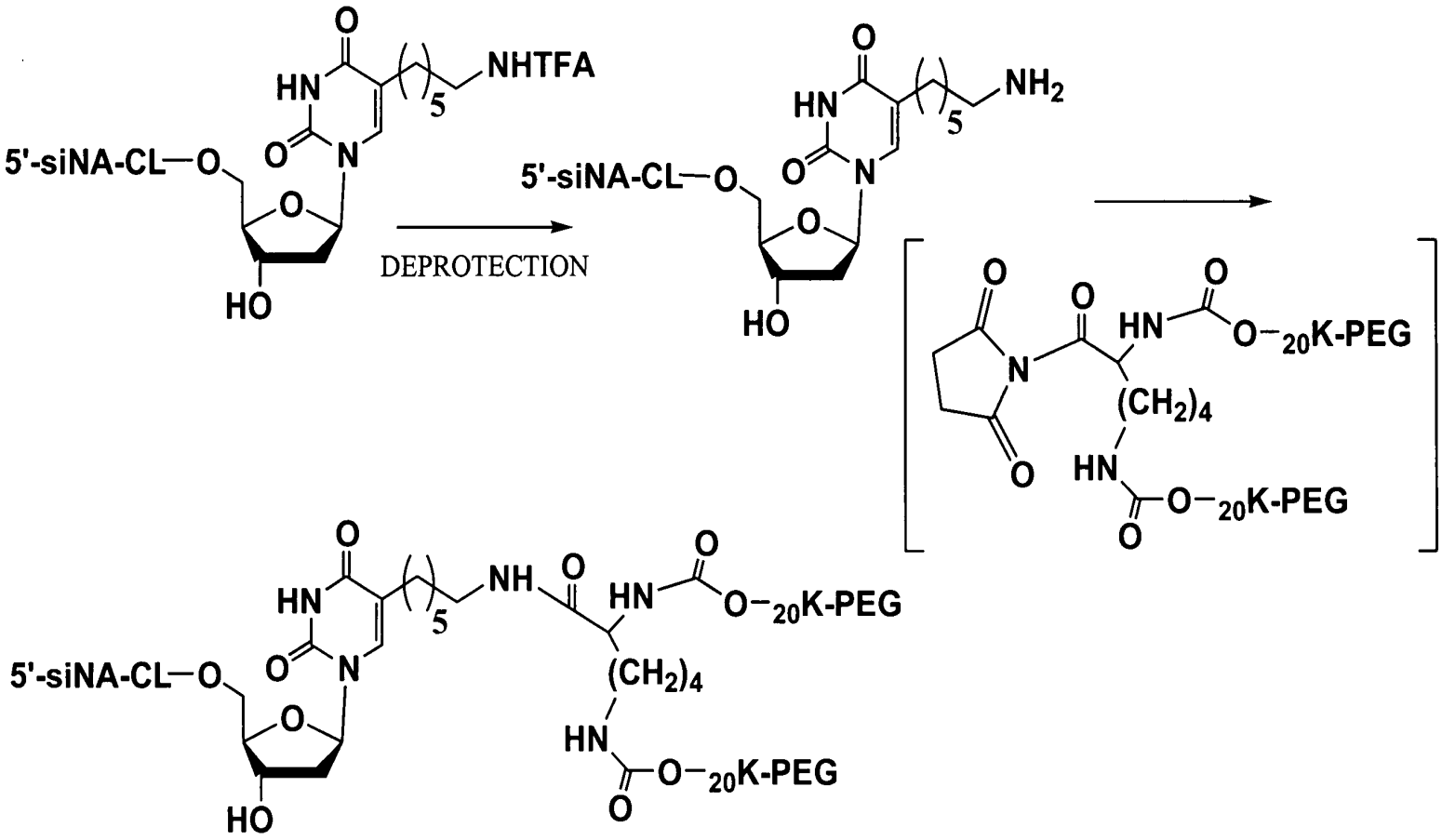
Where n is an integer from 1 to 20

Figure 54: siNA Cholesterol Conjugate



CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT

Figure 55: siNA 3'-PEG Conjugate



CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER THAT IS OPTIONALLY PRESENT

Figure 56: siNA 3'-Cholesterol Conjugate

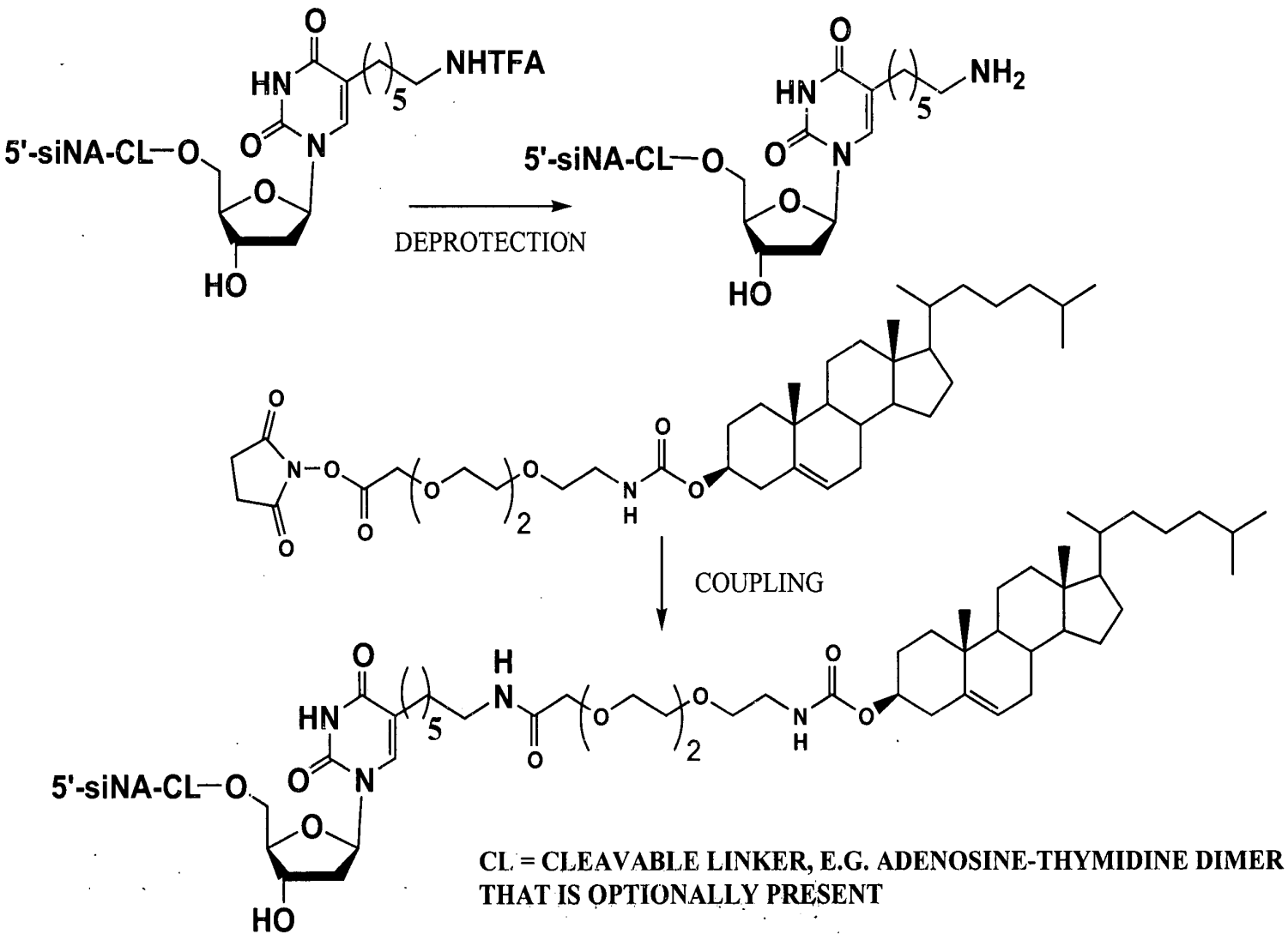
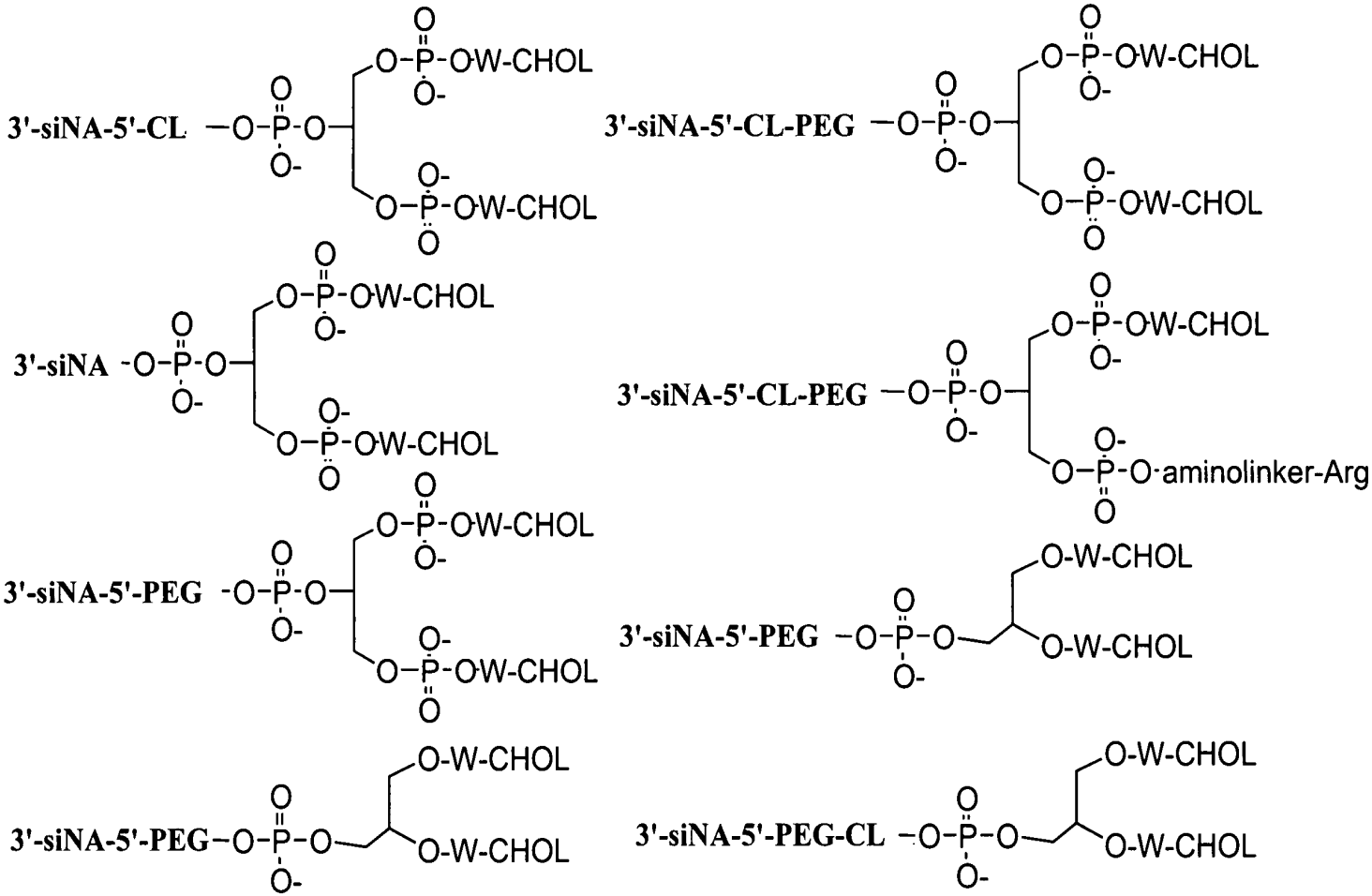
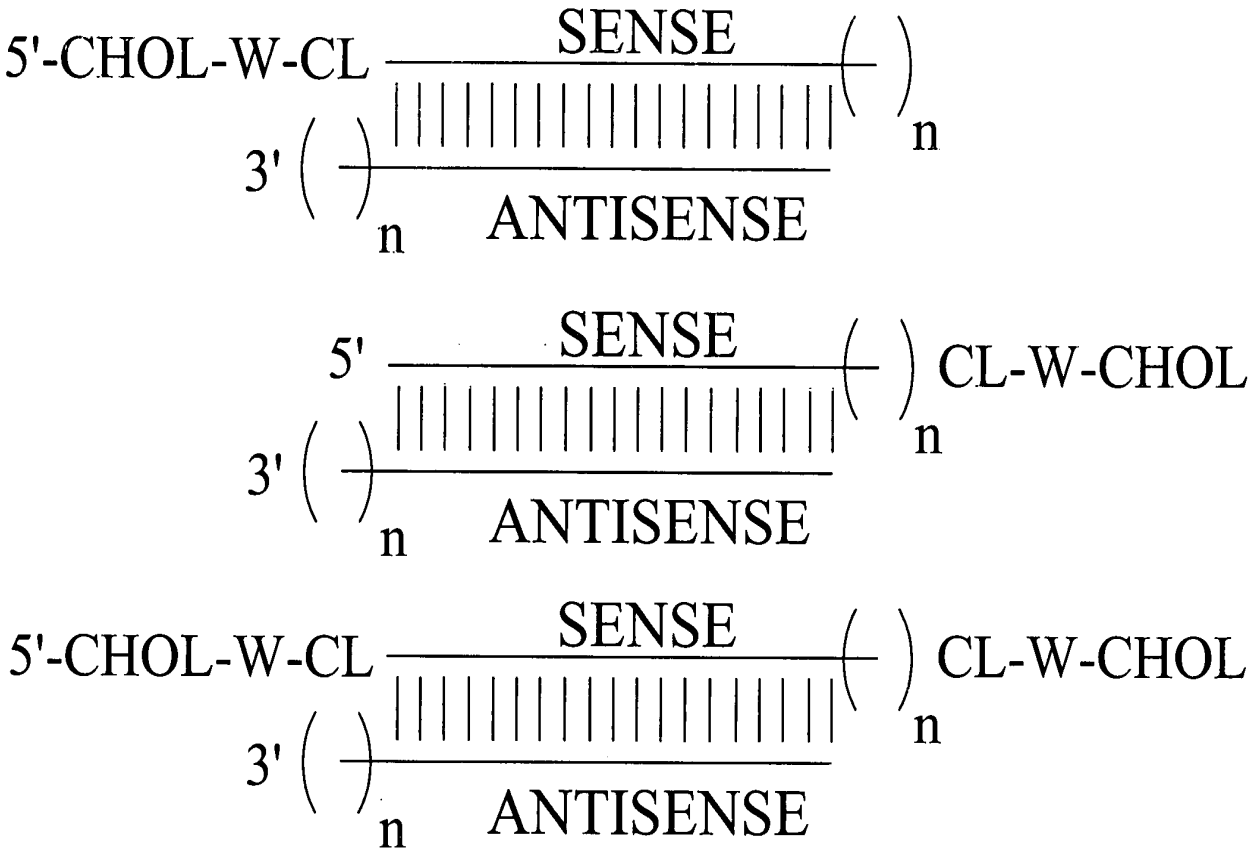


Figure 57: Nucleic Acid Cholesterol Conjugates



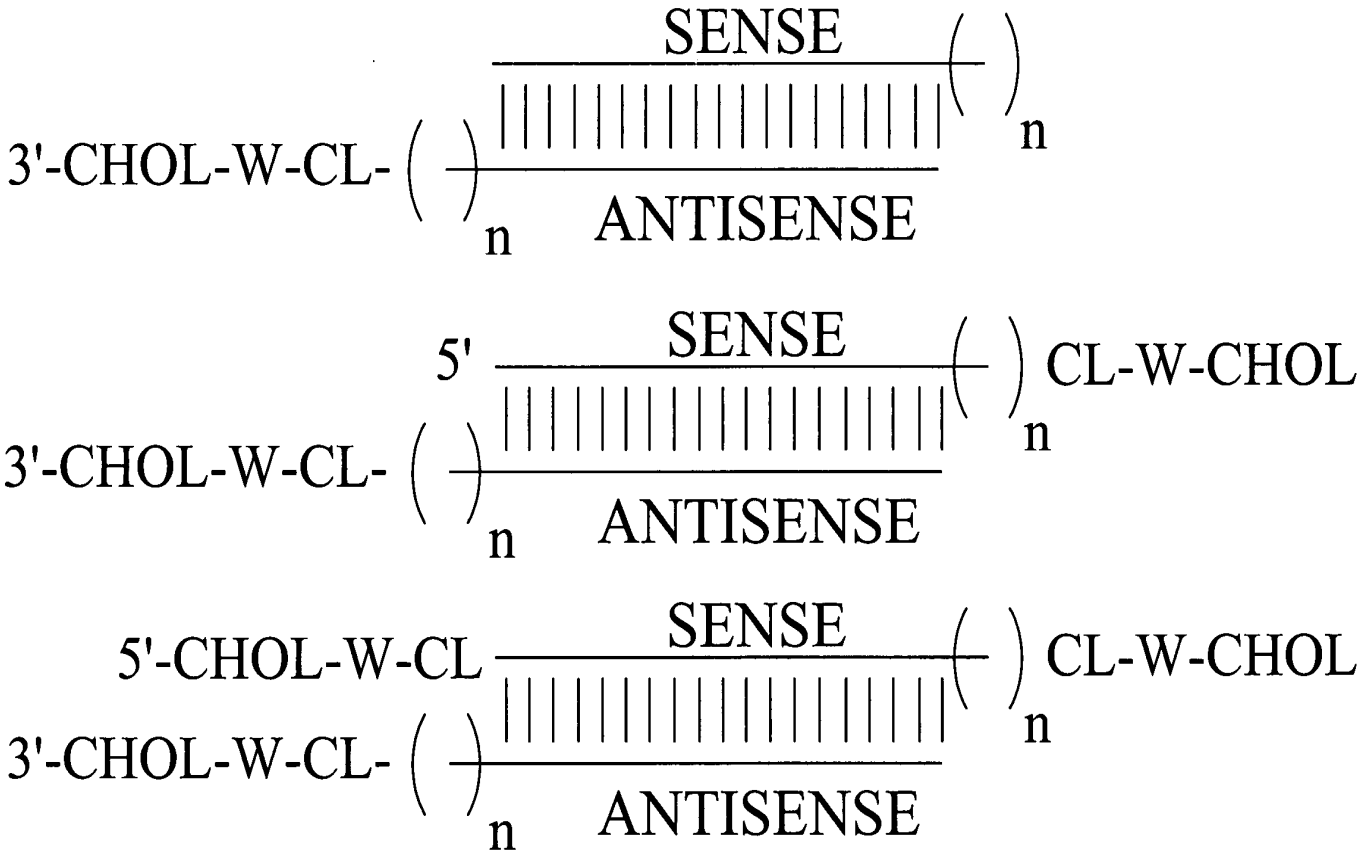
PEG=polyethylene glycol
CL=cleavable linker (e.g. A-dT, C-dT)
siNA= short interfering nucleic acid molecule or a portion thereof
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 109 or 112)

Figure 58: siNA Cholesterol Conjugates



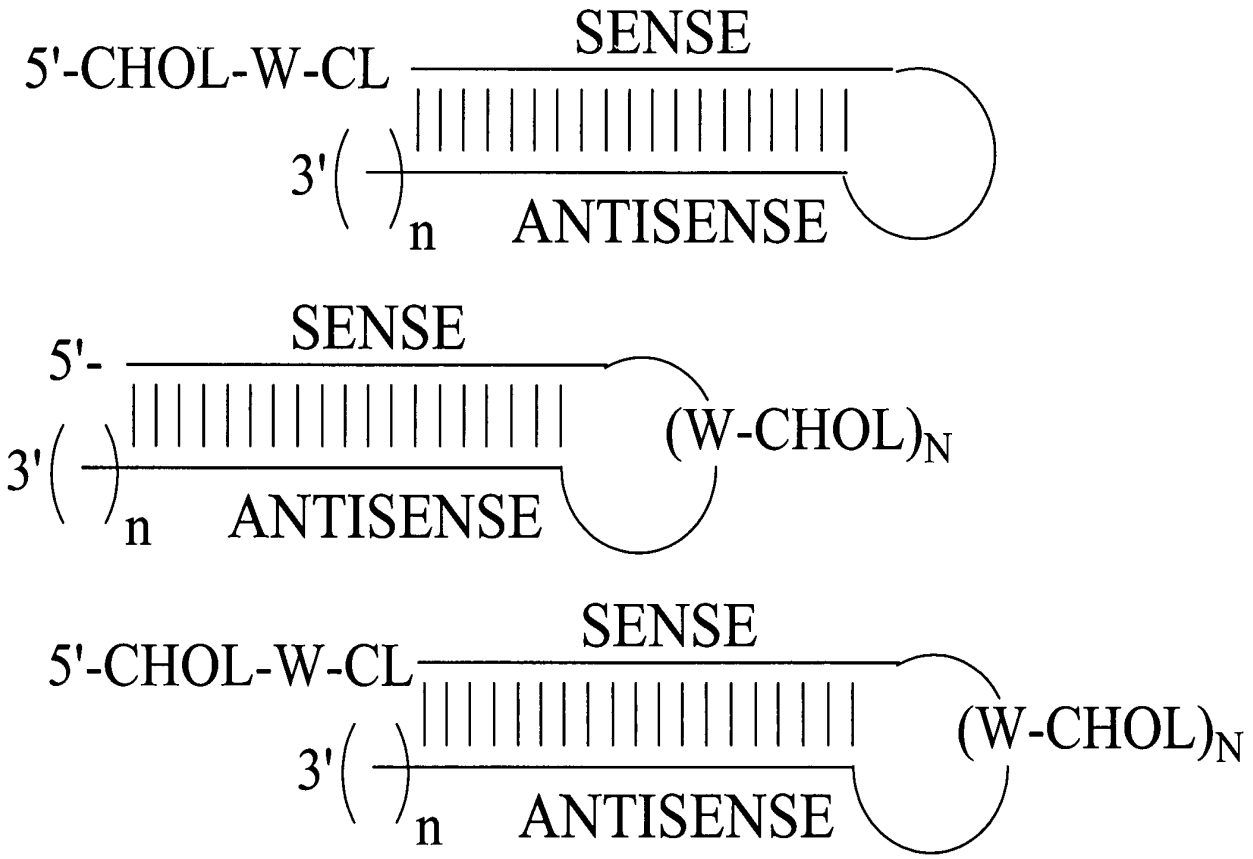
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 107, 108, 109 or 115)
n = integer, e.g. 1, 2, or 3

Figure 59: siNA Cholesterol Conjugates



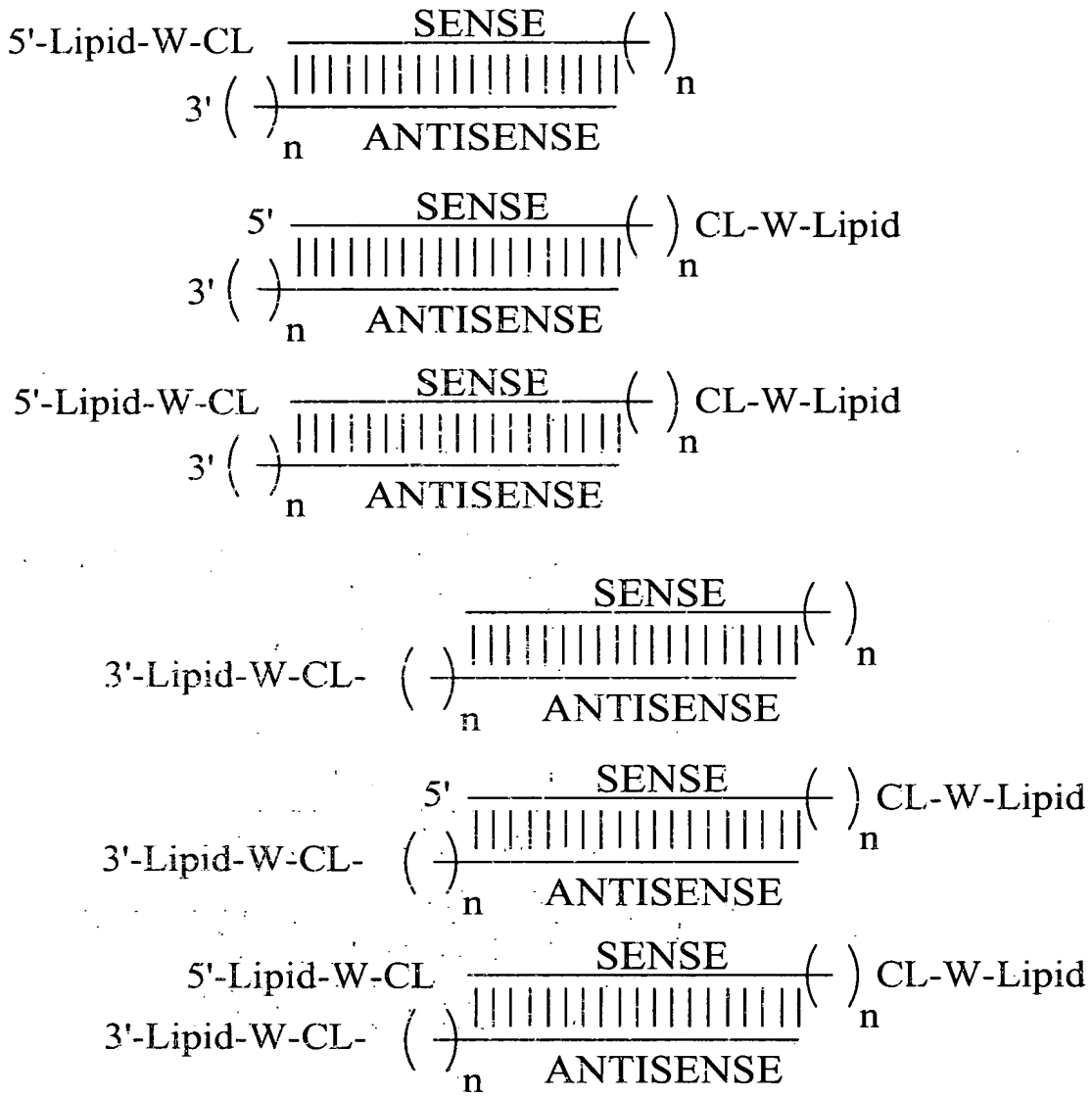
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 107, 108, 109 or 115)
n = integer, e.g. 1, 2, or 3

Figure 60: siNA Cholesterol Conjugates



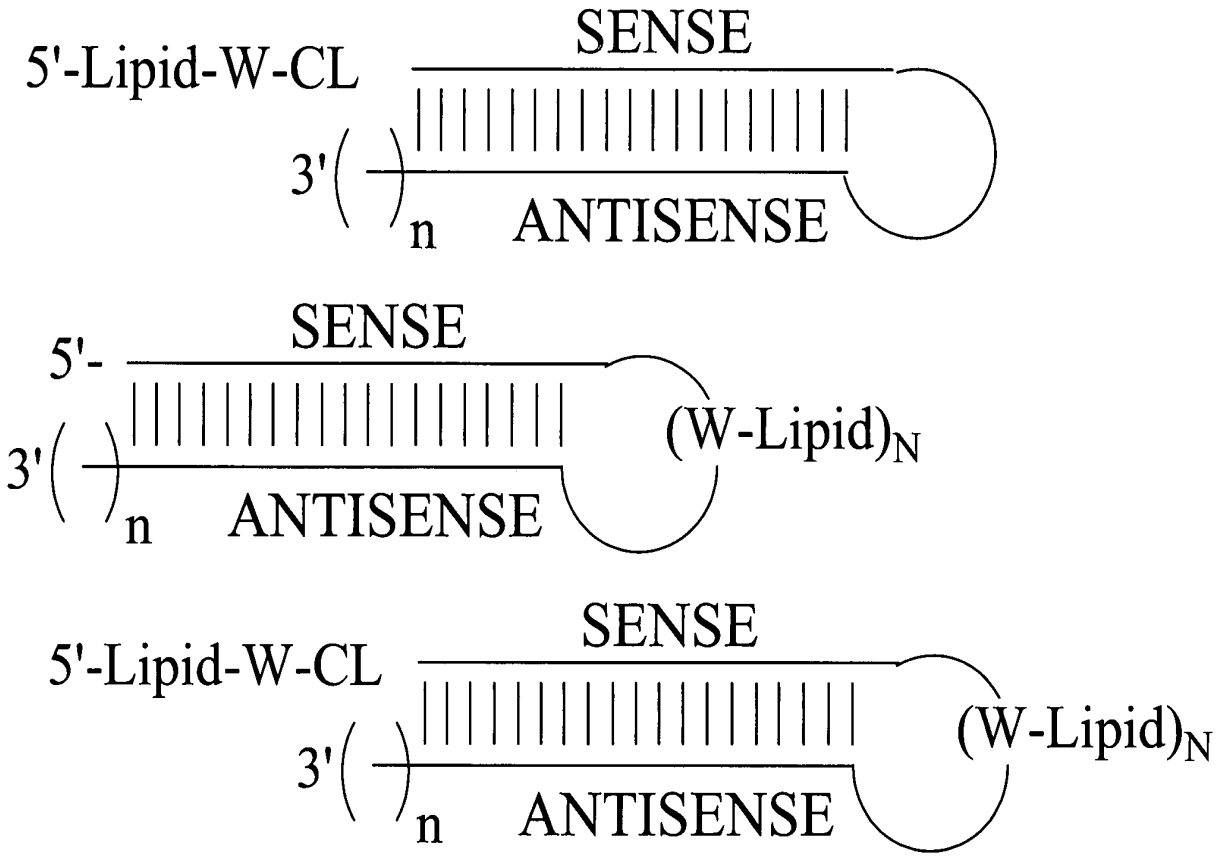
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 107, 108, 109 or 112)
n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

Figure 61: siNA Lipid Conjugates



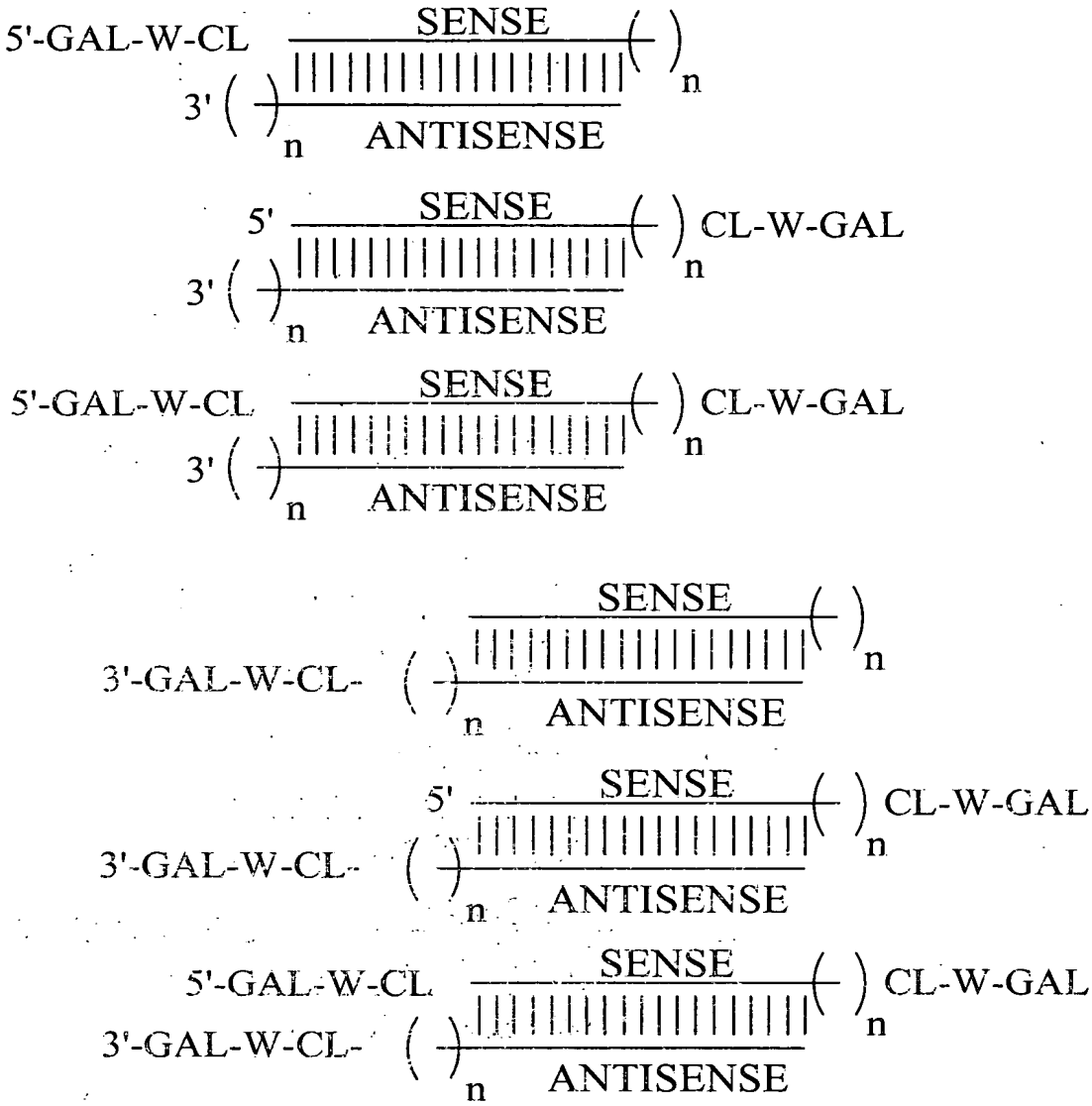
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
Lipid=Straight chain or branched alkyl or fatty acid, e.g. $C_{18}H_{37}$
W= linker molecule (see for example Formulae 48, 49, 64, or 65)
n = integer, e.g. 1, 2, or 3

Figure 62: siNA Lipid Conjugates



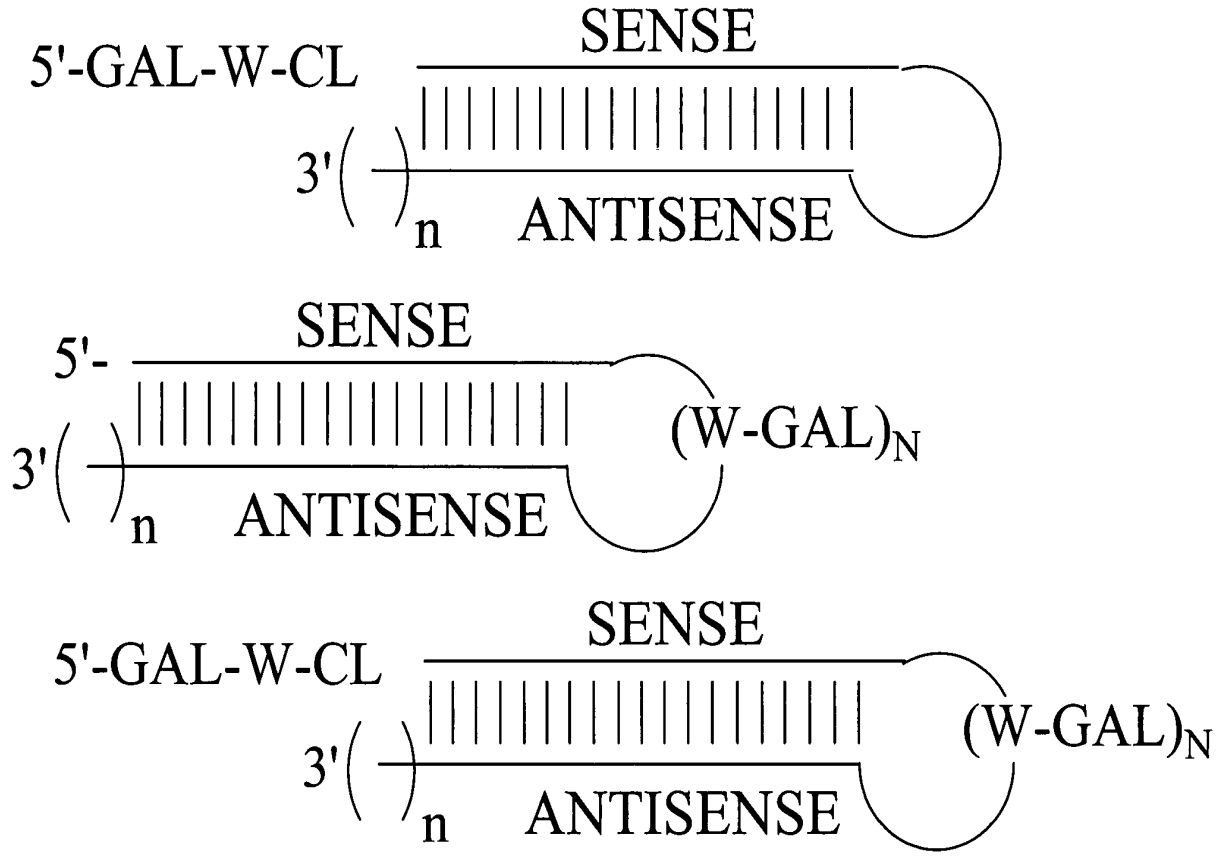
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
Lipid=Straight chain or branched alkyl or fatty acid, e.g. $C_{18}H_{37}$
W= linker molecule (see for example Formulae 48, 49, 64, or 65)
n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

Figure 63: siNA Galactosamine Conjugates



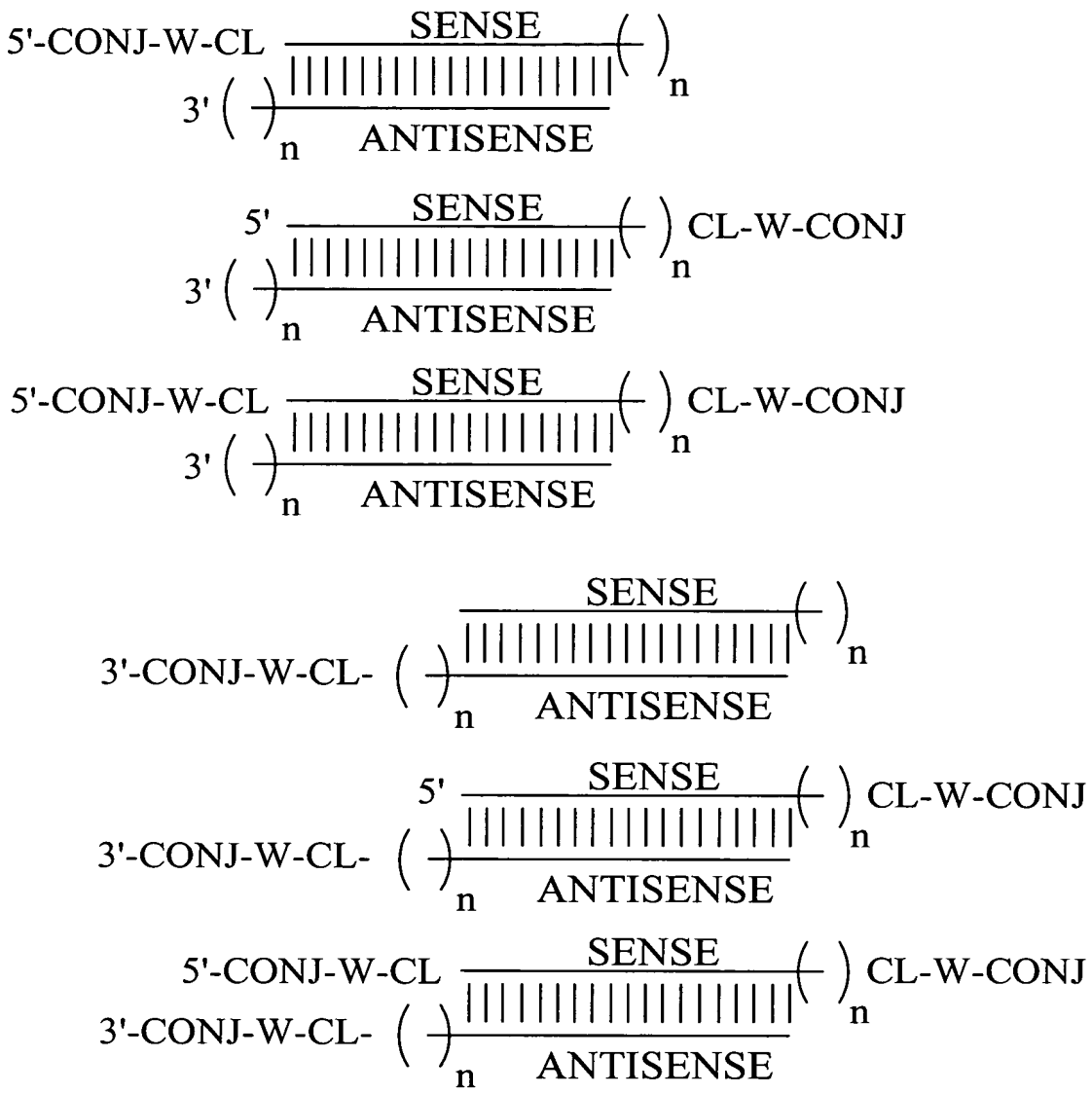
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106
W= linker molecule (see for example Formulae 102 or 103)
n = integer, e.g. 1, 2, or 3

Figure 64: siNA Galactosamine Conjugates



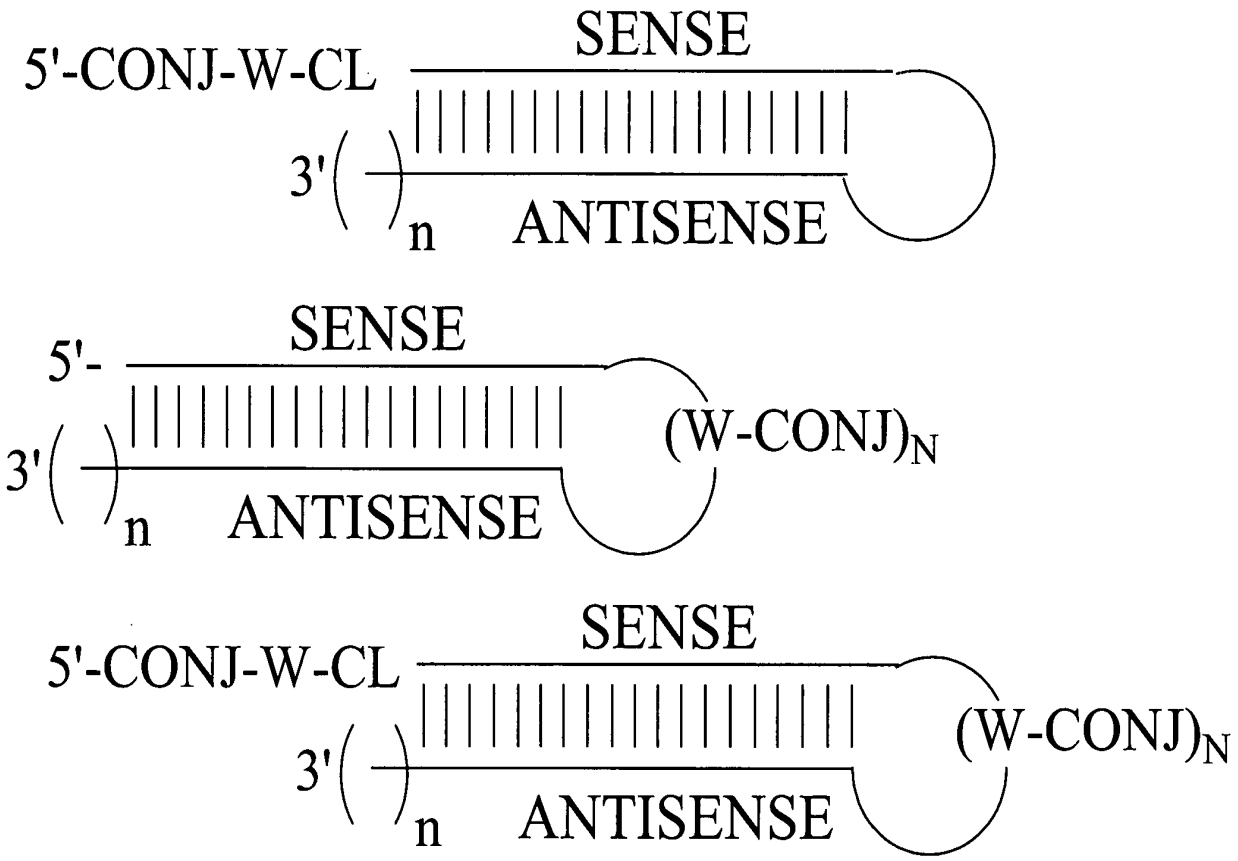
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106
W= linker molecule (see for example Formulae 102 or 103)
n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

Figure 65: Generalized siNA Conjugate Design



CONJ=any biologically active molecule or conjugate as described herein
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
W= linker molecule
n = integer, e.g. 1, 2, or 3

Figure 66: Generalized siNA Conjugate design



CONJ=any biologically active molecule or conjugate as described herein
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
W= linker molecule
n = integer, e.g. 1, 2, or 3
N=integer, e.g. 1, 2, 3, or 4

Figure 67: Distribution of Intact siNA in Liver After SC Administration of Conjugated or Unconjugated Chemistries

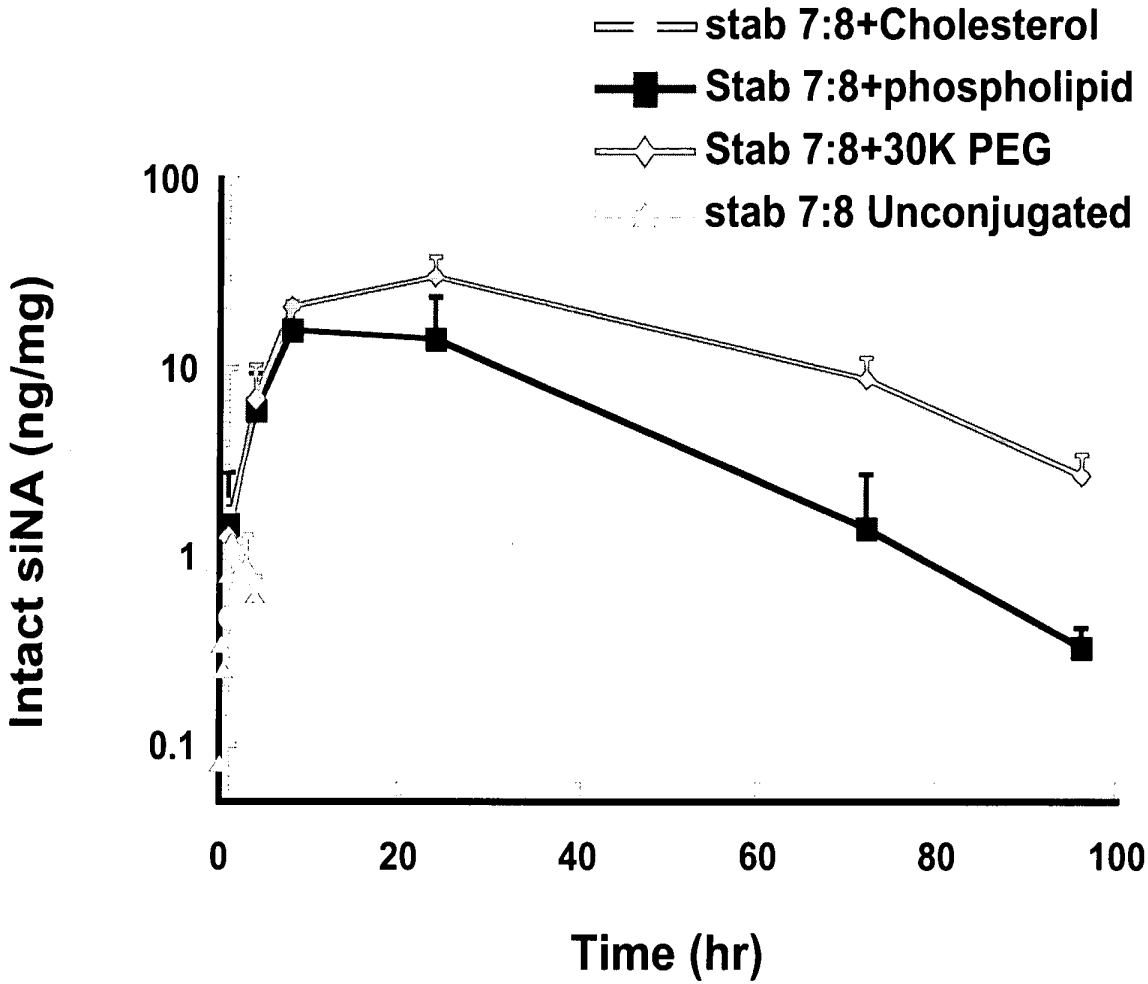


Figure 68: Lipid Free Delivery of HBV siNA Conjugates in Cell Culture

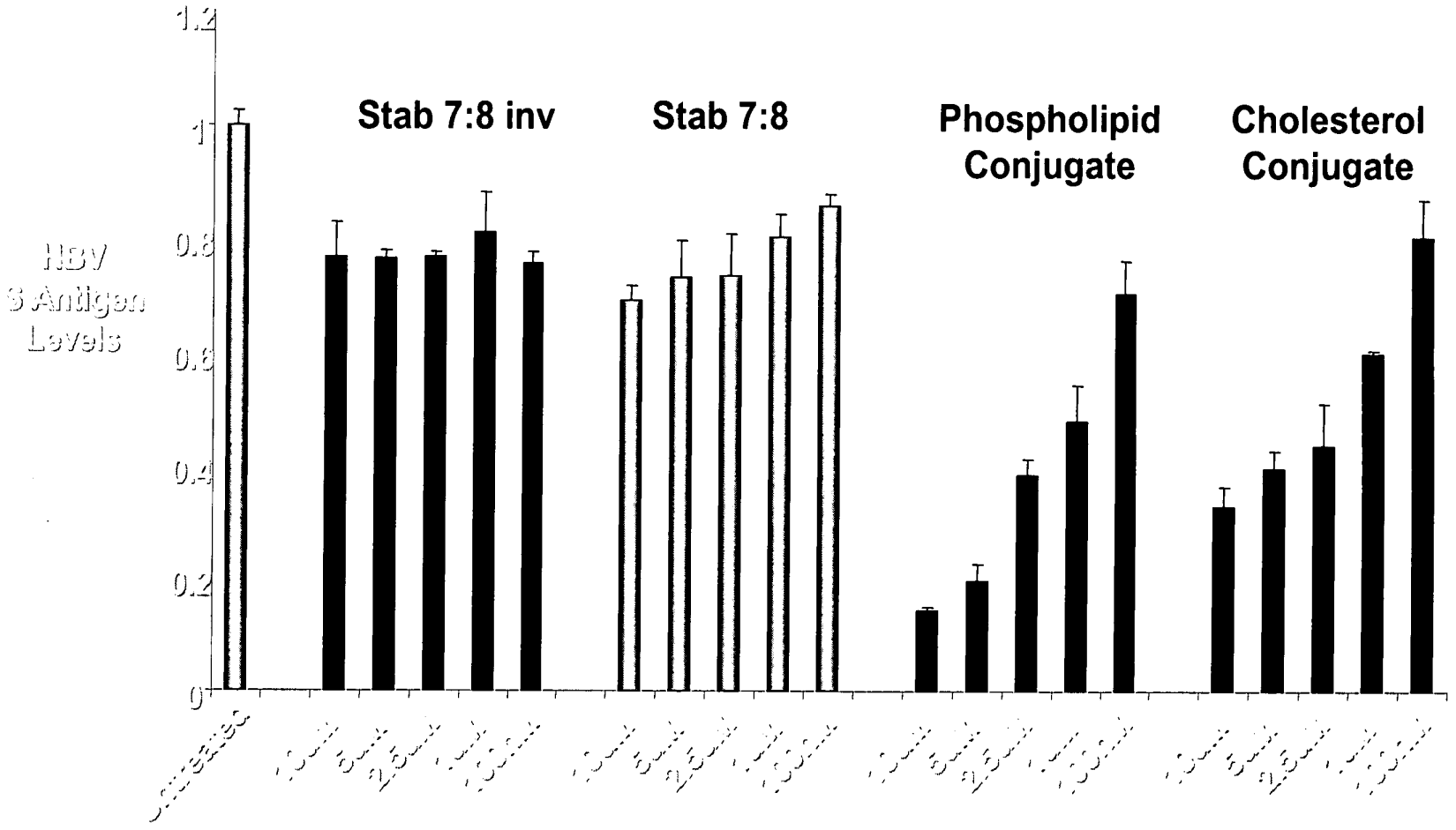


Figure 69: Scale-up of "mono" Galactosamine phosphoramidite

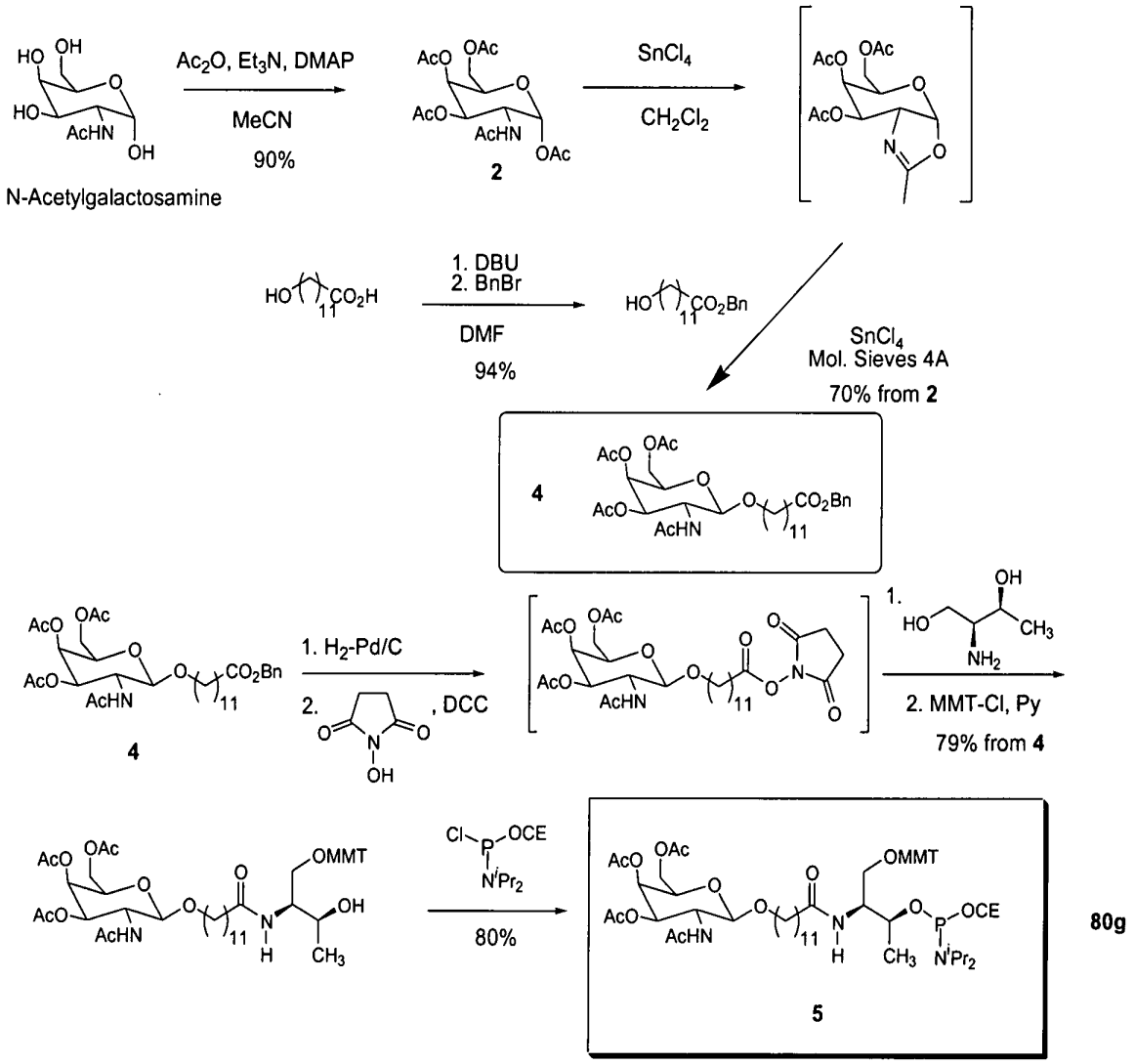


Figure 70: Synthesis of "tri" Galactosamine phosphoramidite

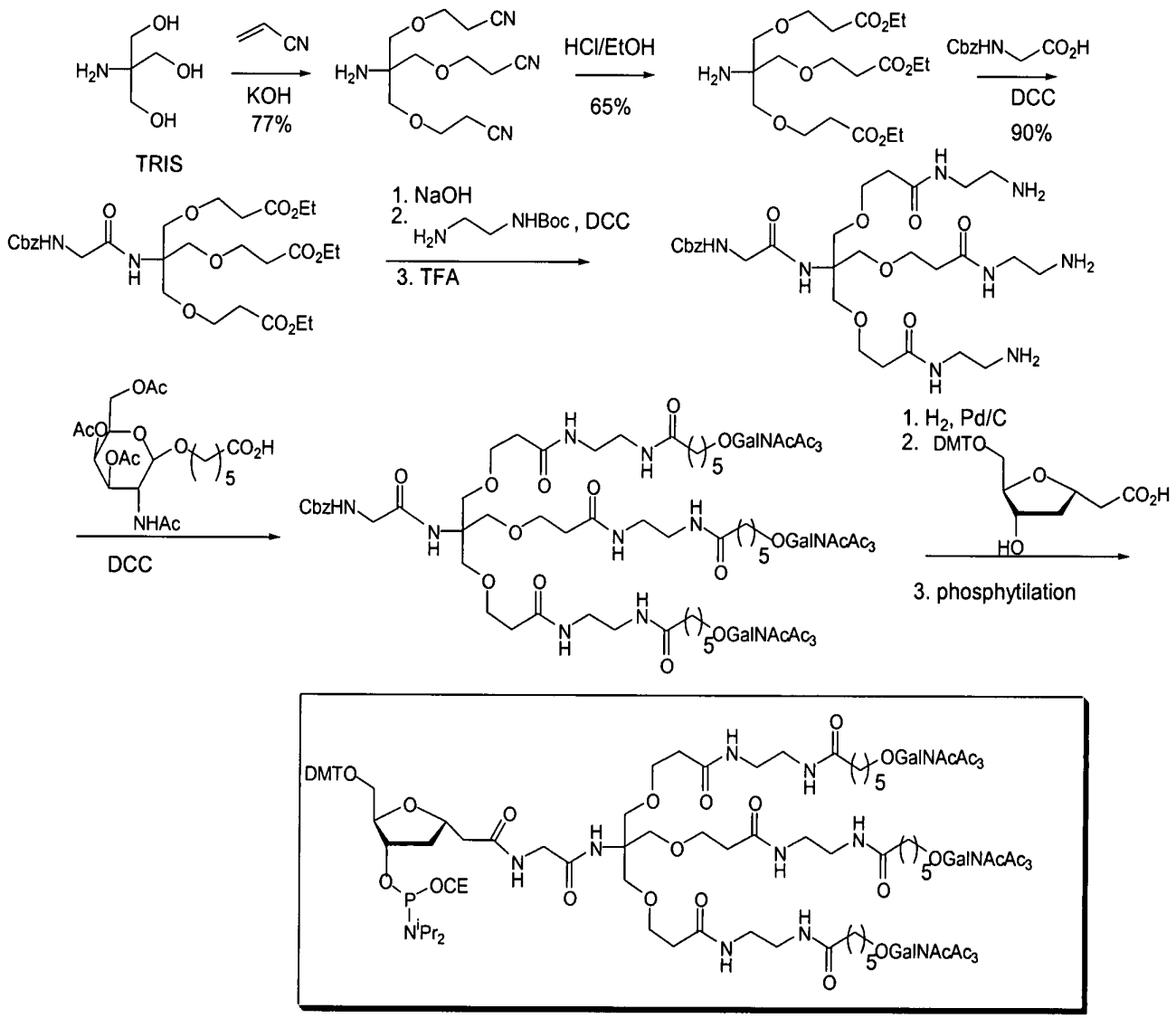


Figure 71: Synthesis of another Tri-Galactosamine Conjugate

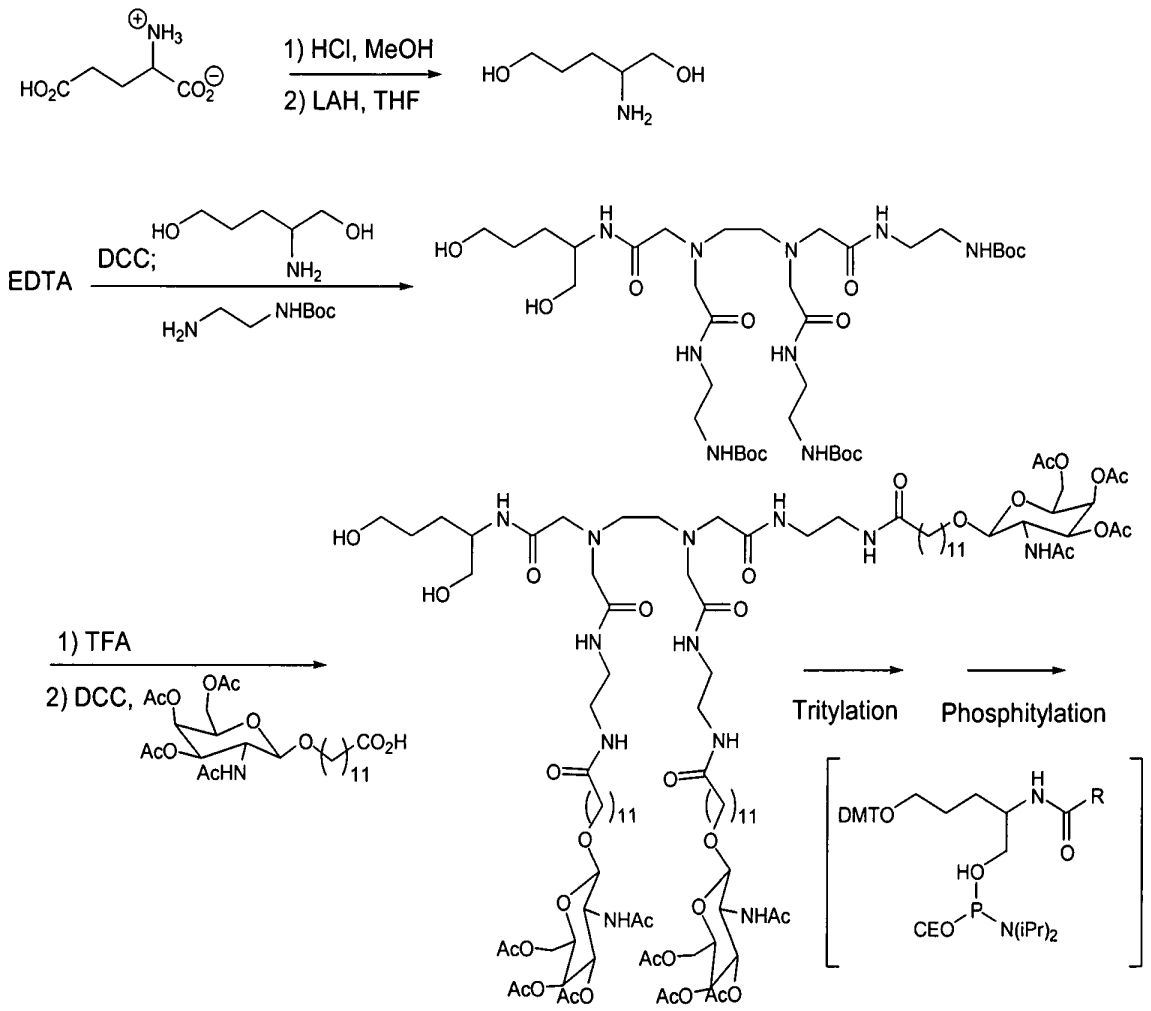


Figure 72: Alternate Synthesis of Tri-Galactosamine Conjugate

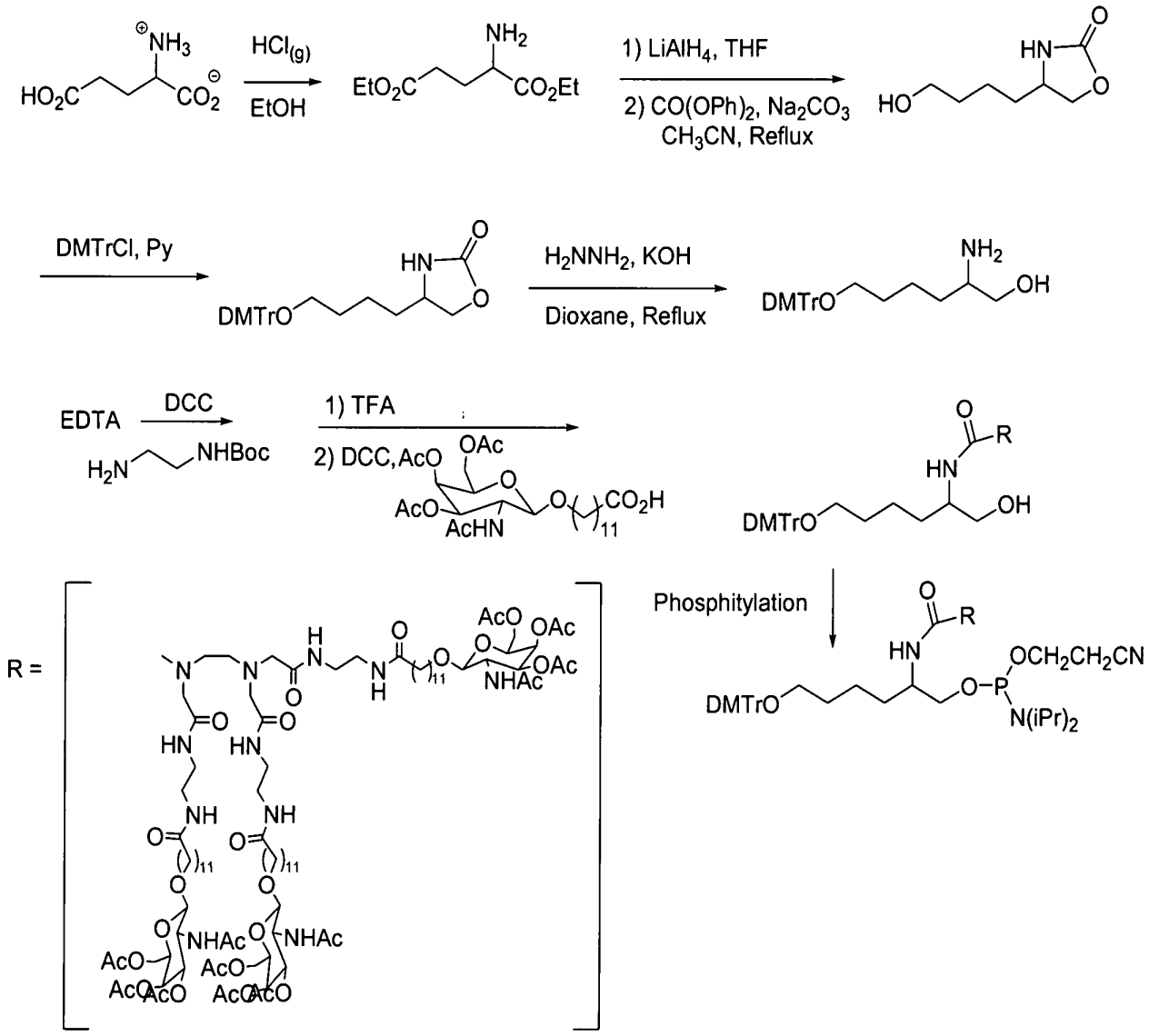


Figure 73: Synthesis of NHS Cholesterol Conjugate

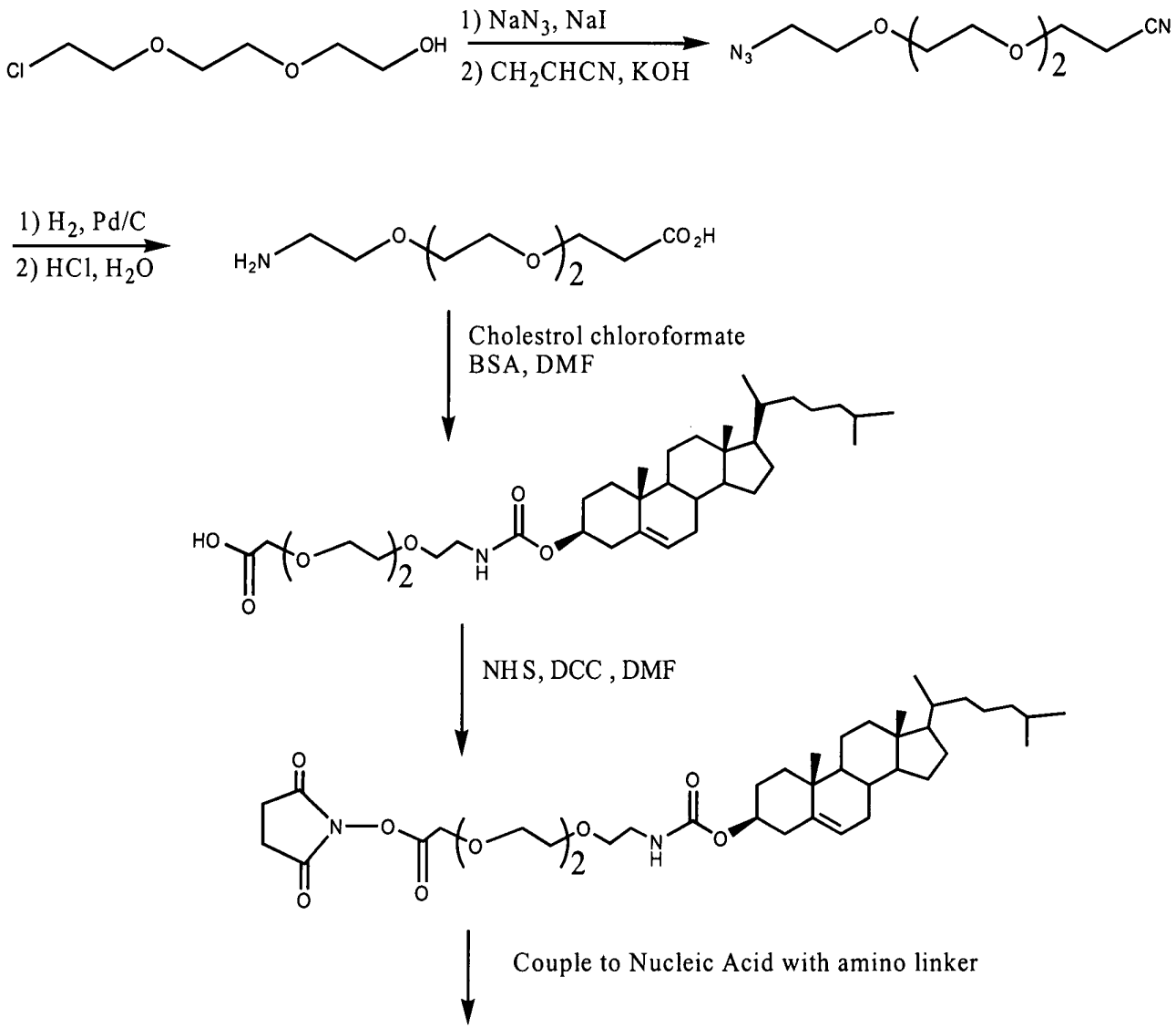
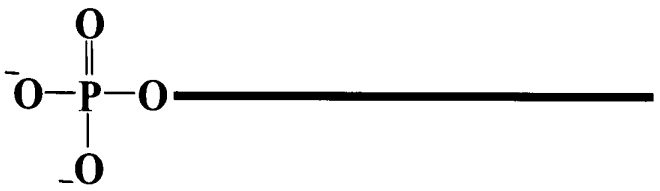
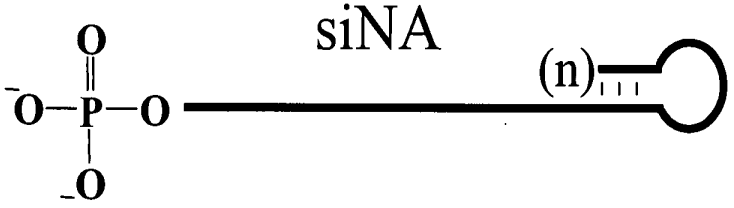


Figure 74: Phosphorylated siNA constructs

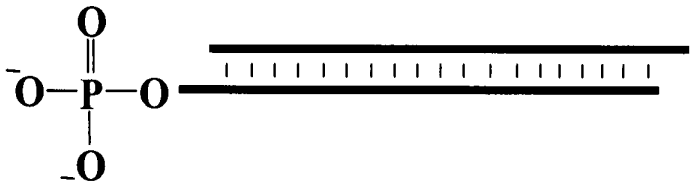


Phosphates can be modified as described herein

Asymmetric hairpin siNA



Asymmetric duplex siNA



(n) = number of base pairs (e.g. 3-18 bp)

Figure 75: 5'-phosphate modifications

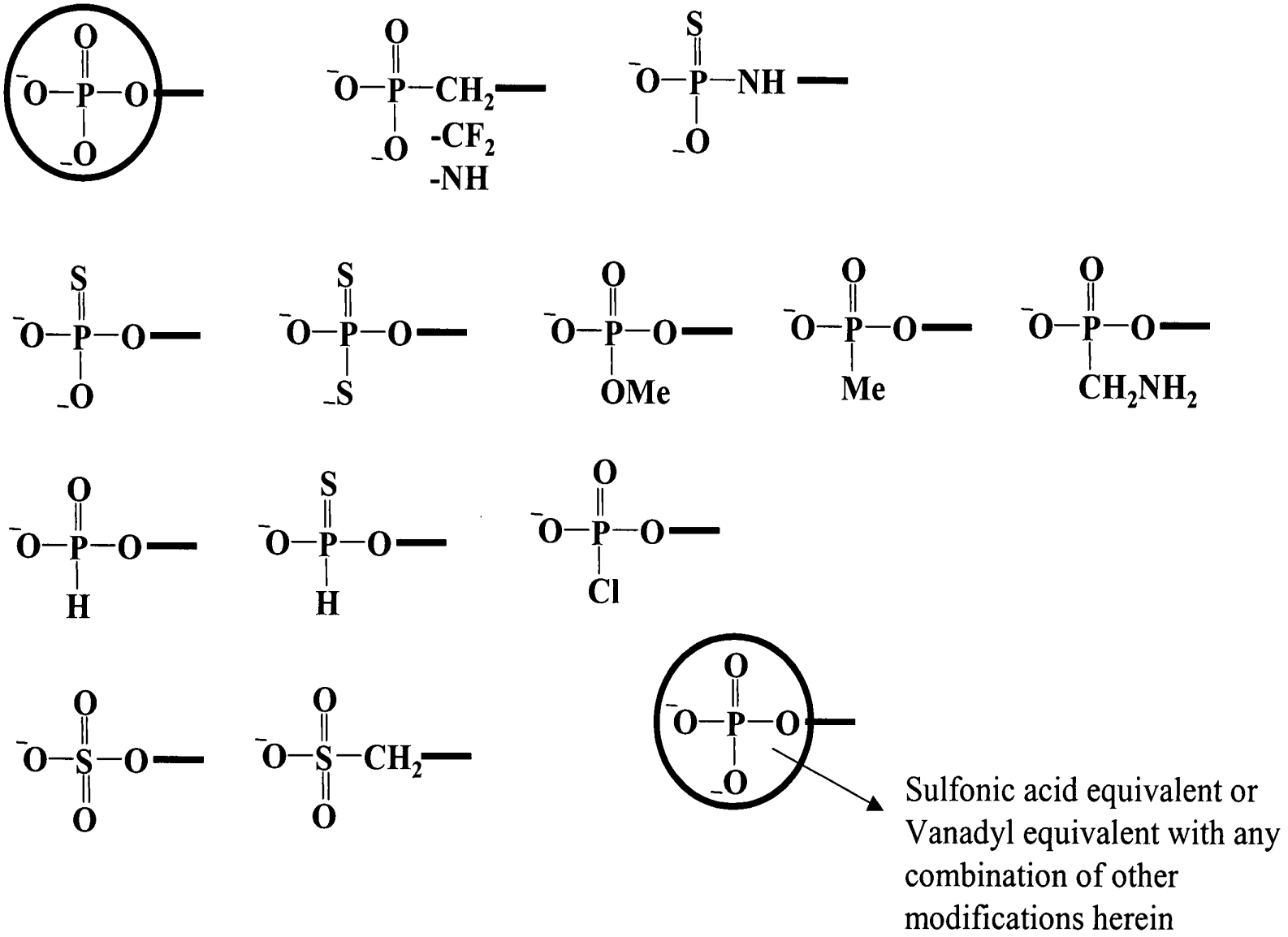


Figure 76: siNA Targeting VEGFR-1 Inhibits VEGF-Induced Rat Corneal Angiogenesis

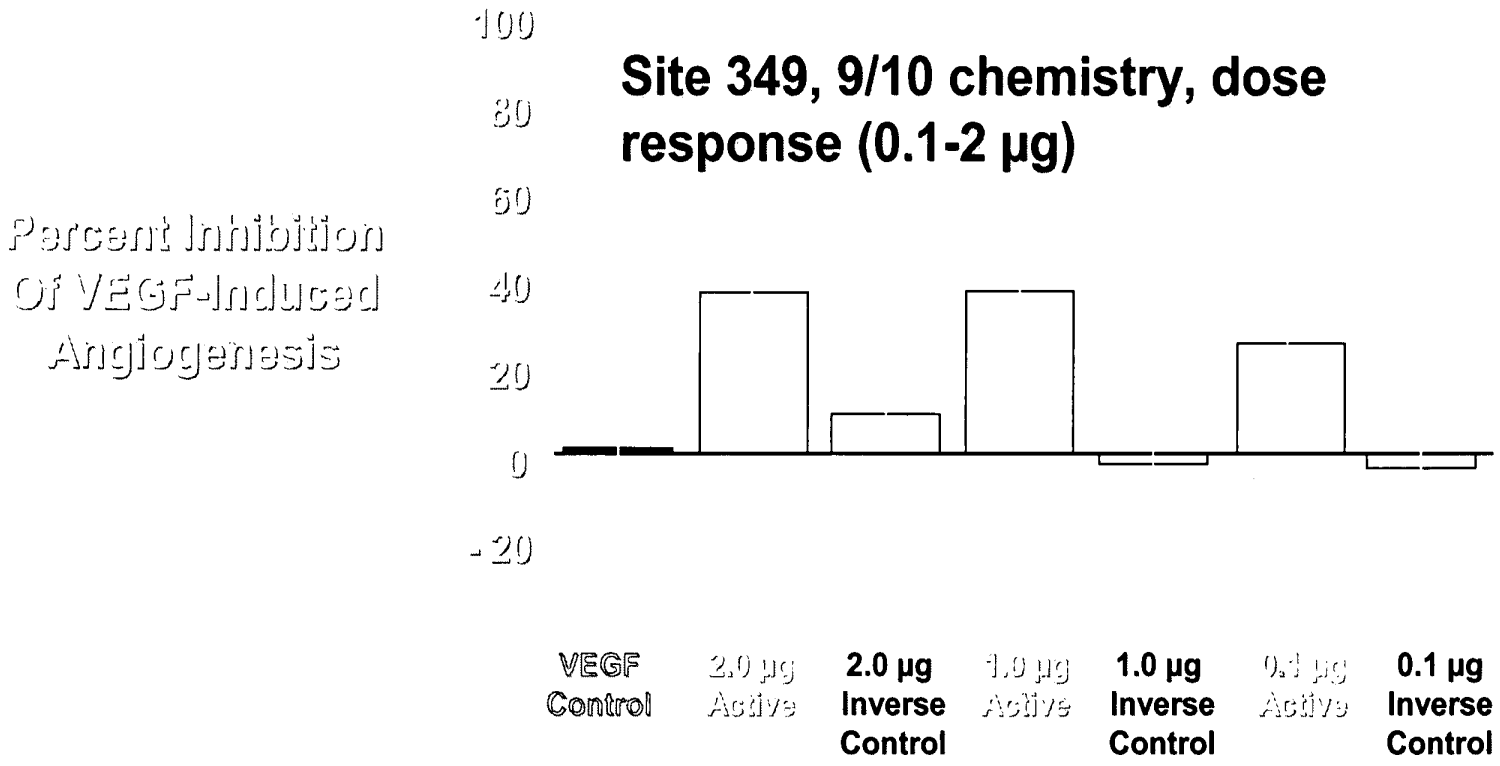


Figure 77: Duration of Effect of Modified siNA Constructs

A

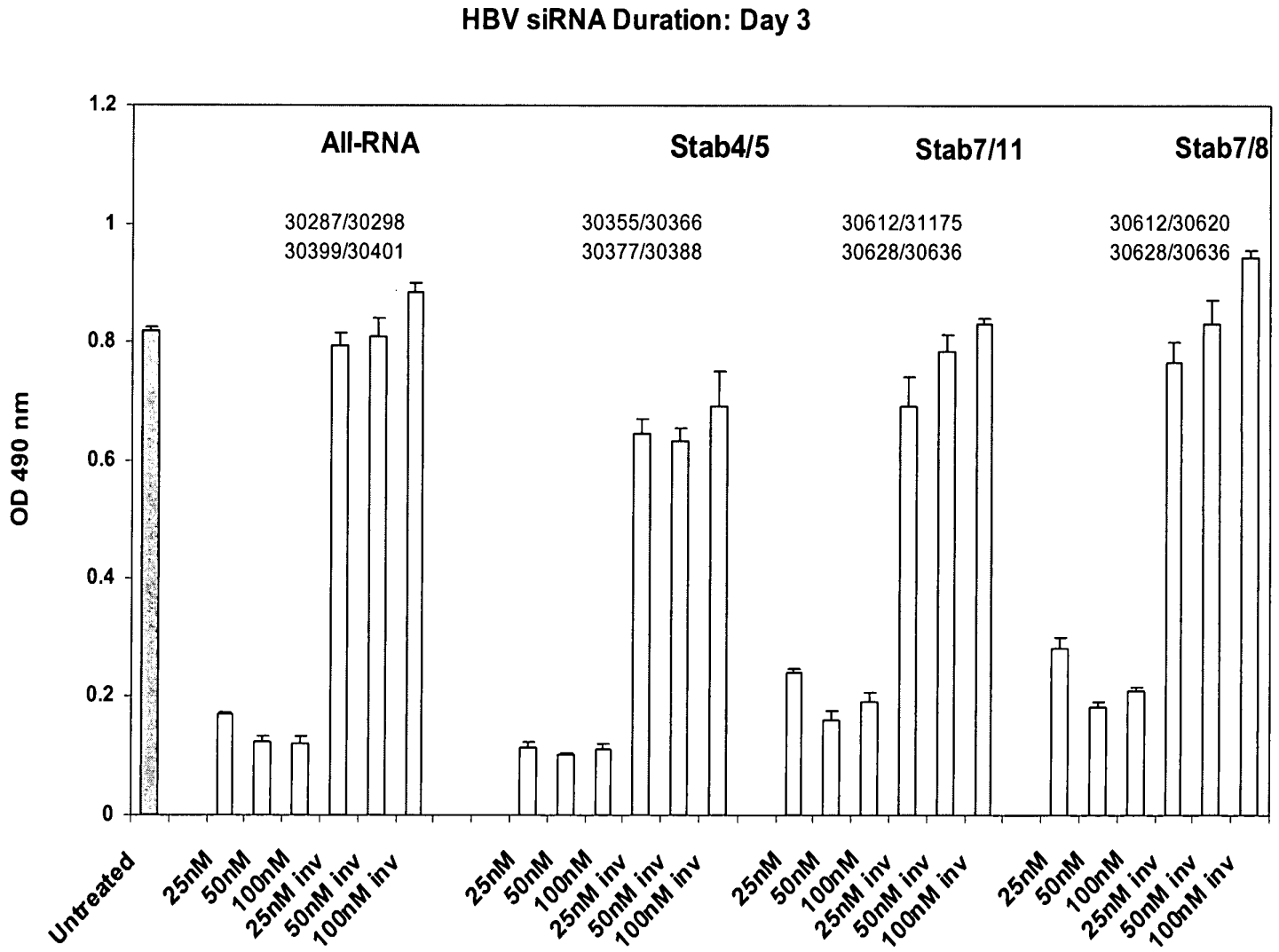


Figure 77: Duration of Effect of Modified siNA Constructs

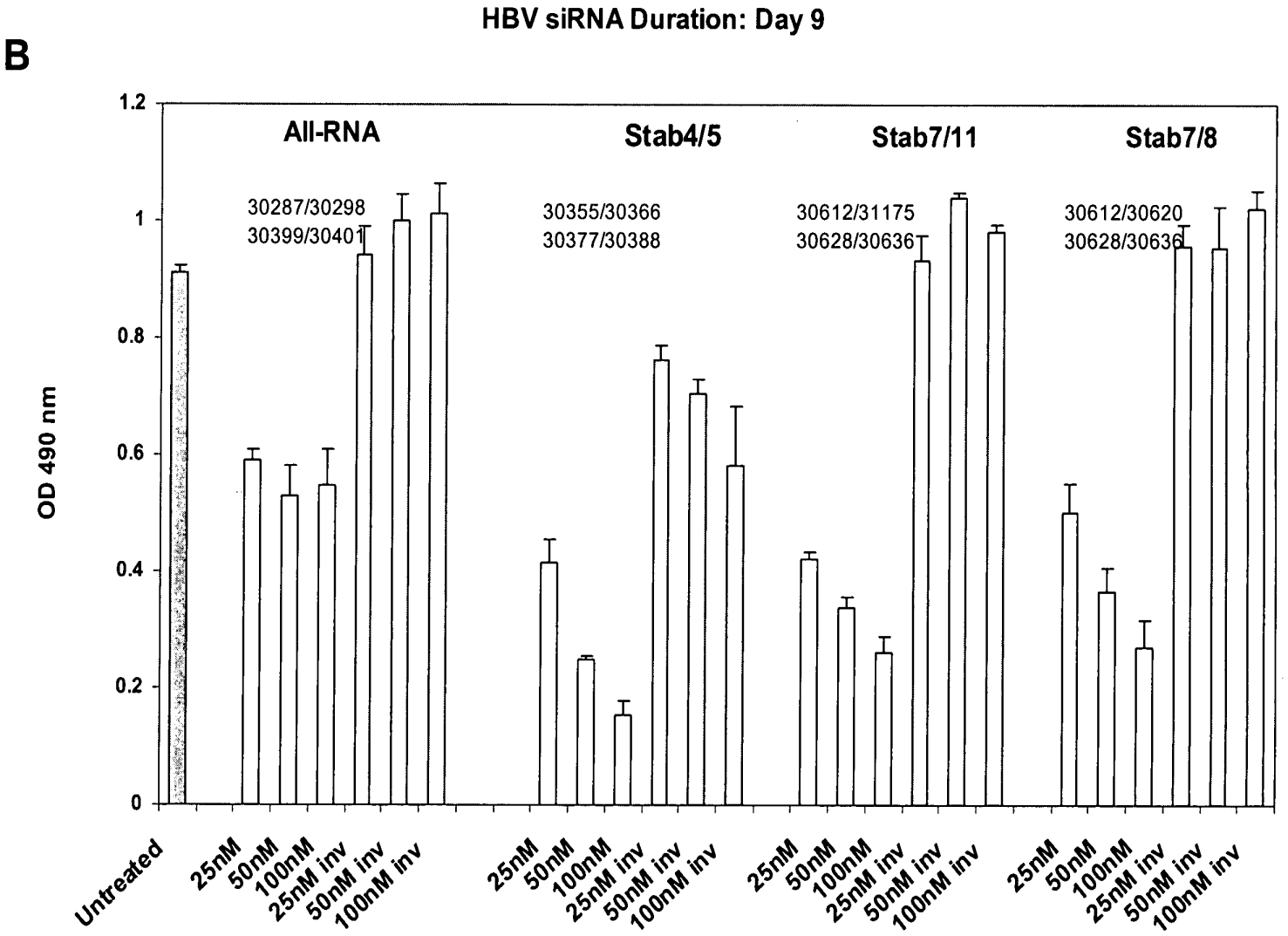


Figure 77: Duration of Effect of Modified siNA Constructs

HBV siRNA Duration: Day 21

C

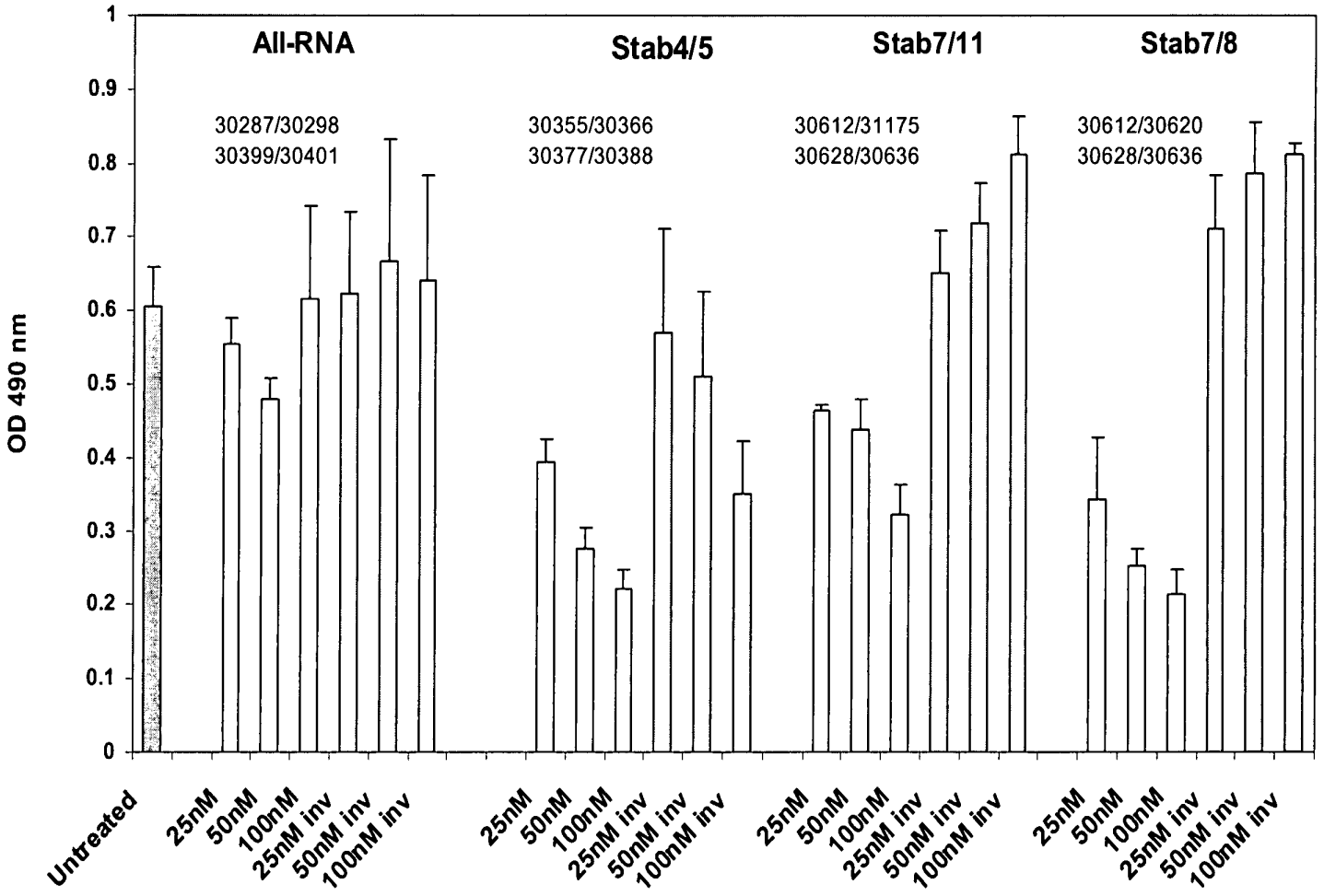


Figure 77: Duration of Effect of Modified siNA Constructs

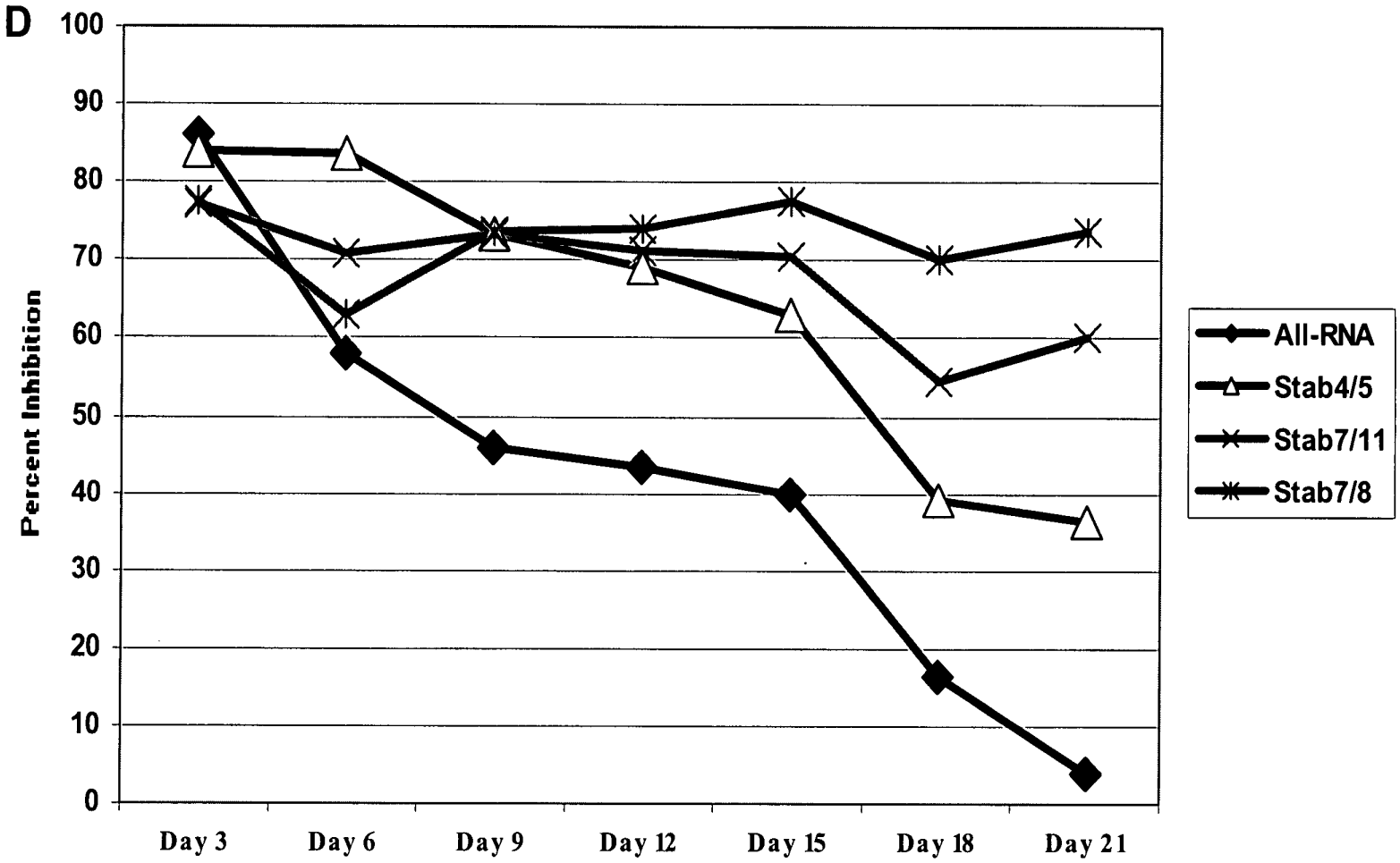


Figure 77: Duration of Effect of Modified siNA Constructs

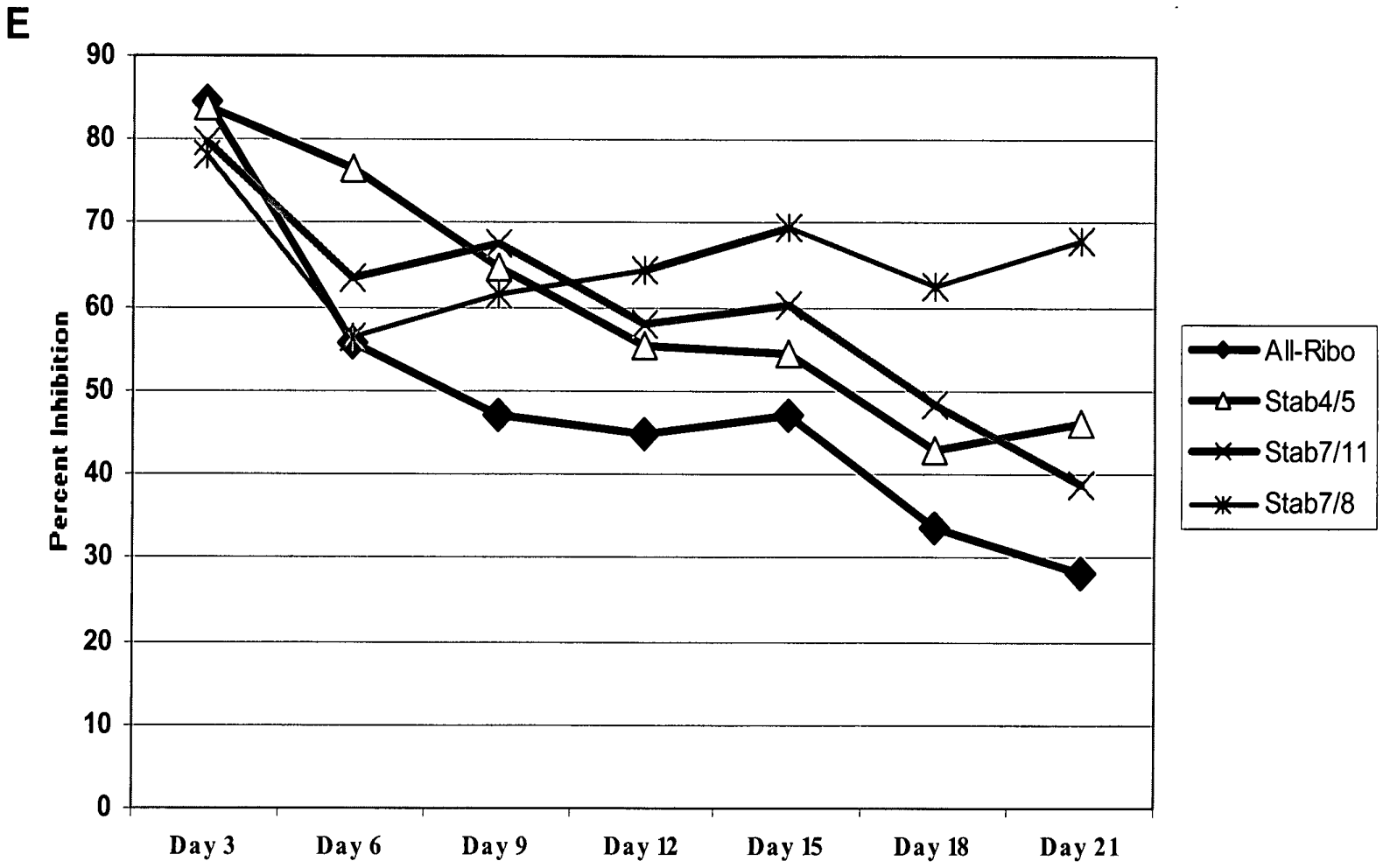


Figure 77: Duration of Effect of Modified siNA Constructs

F

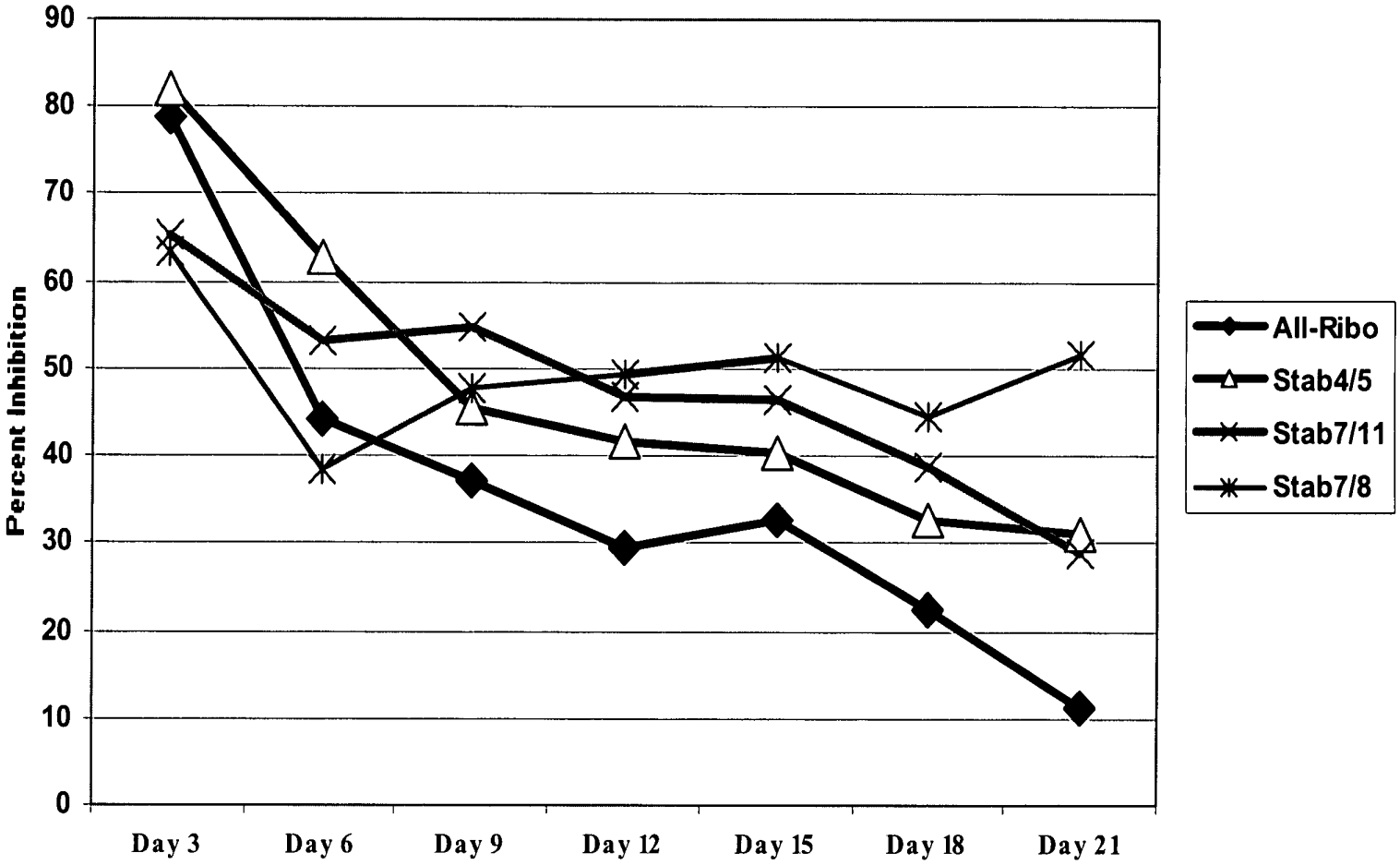
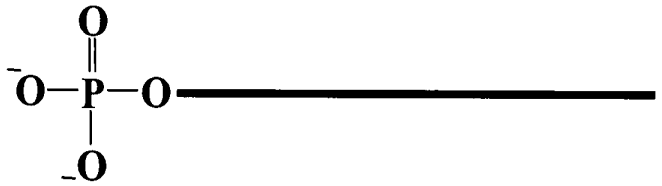
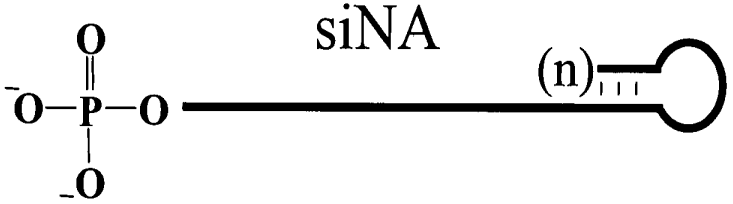


Figure 78: Phosphorylated siNA constructs

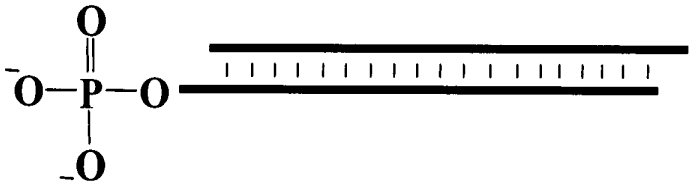


Phosphates can be modified as described herein

Asymmetric hairpin siNA



Asymmetric duplex siNA



(n) = number of base pairs (e.g. 3-18 bp)

Figure 79: 5'-phosphate modifications